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Life cycle and host-parasite relationships of *Khawia iowensis* Calentine and Ulmer, 1961 (Cestoidea: Caryophyllidea)

by

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INTRODUCTION AND HISTORICAL REVIEW

Caryophyllideans are unique among true tapeworms in having a single set of reproductive organs within an unsegmented body and in utilizing aquatic oligochaetes (Annelida) as intermediate hosts. Except for a report by Pearse (1924) of Glaridacris catostomi Cooper, 1920 from the mudpuppy, Necturus maculosus Rafinesque (Amphibia) in Wisconsin, all records of adult caryophyllideans from vertebrates involve freshwater fish (Mackiewicz 1972). Notable exceptions are reports of probable accidental infections of marine fish from coastal brackish waters of the Baltic (Janiszewska 1939). North American caryophyllideans normally utilize cypriniform fish of two families (Catostomidae and Cyprinidae) as definitive hosts. Mackiewicz (1972) reviewed the normal and accidental fish hosts of caryophyllideans. Gravid caryophyllideans may range in length from 0.85 (Balanotaenia newguinensis Mackiewicz and Blair, 1978) to 170.0 mm (Khawia sinensis Hsü, 1935).

The zoogeographical distribution of caryophyllideans includes the Nearctic, Palearctic, Ethiopian, Oriental and Australian regions. There are no published records from Neotropical fish. Palearctic caryophyllideans are found primarily in fish of the family Cyprinidae. Those of the Ethiopian, Oriental and Australian regions normally use fish

of several siluriform families as final hosts.

According to Hunter (1930), tapeworms now recognized as caryophyllideans were first described by Pallas in 1781 as Taenia laticeps. This worm was later placed in the genus Caryophyllaeus Gmelin, 1790. A complete historical treatment of caryophyllideans is not included in this dissertation but the considerable literature dealing with their nomenclature and taxonomic relationships has been thoroughly reviewed by Calentine (1963) and by Mackiewicz (1972). Taxonomically, caryophyllideans have been variously linked with the cestodarians, cyathocephalideans and pseudophyllideans (Wardle and McLeod 1952).

Hunter (1927, 1929, 1930) considered caryophyllideans as a family (Caryophyllaeidae) of the order Pseudophyllidea Carus, 1863 and was apparently the first author to divide the group into smaller taxa. He distinguished four subfamilies: Caryophyllaeinae Carus, 1863; Capingentinae Hunter, 1930; Lytocestinae Hunter, 1927 and Wenyoninae Hunter, 1927.

Hyman (1951) also treated caryophyllideans as a family of the Pseudophyllidea, but Wardle and McLeod (1952) included them as a new order of the class Cestoda Monticelli, 1892 and elevated Hunter's four subfamilies to familial rank. Yamaguti (1959) retained the order Caryophyllidea and as Calentine (1963) points out, not only inconsistently placed it (p. 7) within the subclass Eucestoda Southwell, 1930 but

also included it (pp. 8, 18 and 451) within the subclass Cestodaria Monticelli, 1892. Yamaguti recognized a single family, Caryophyllaeidae, containing three subfamilies: Caryophyllaeinae, Capingentinae and Lytocestinae. Tapeworms of the genus Wenyonia Woodland, 1923 were included within the Caryophyllaeinae.

Joyeux and Baer (1961), Kulakovskaya (1961) and Stunkard (1962) all considered caryophyllideans as a family within the order Pseudophyllidea. Bykhovskaya-Pavlovskaya et al. (1962), Hoffman (1967), Schmidt (1970) and Mackiewicz (1972) recognized caryophyllideans as belonging to the order Caryophyllidea (class Cestoidea Rudolphi, 1808, subclass Cestoda or Eucestoda). Bykhovskaya-Pavlovskaya et al. (1962) attributed establishment of the term Caryophyllidea to Van Beneden, as did Mackiewicz (1972). Calentine (1963) pointed out that Carus latinized Van Beneden's "Caryophyllé" as Caryophyllidea and that Mola (1929) had been the first to use it at an ordinal rank.

Wardle, McLeod and Radinovsky (1974) proposed the class Cotyloda for the six orders they regarded as pseudotapeworms (Caryophyllidea, Pseudophyllidea, Gyrocotylidea Poche, 1926, Amphilinidea Poche, 1922, Spathebothridea Wardle and McLeod, 1952 and Diphyllidea Wardle, McLeod and Radinovsky, 1974). The remaining groups were placed in the class Eucestoda.

In addition, these authors resurrected the Wenyonidae as one of four caryophyllidean families.

Criteria for the identification of subfamilies [or families if one accepts conclusions by Wardle and McLeod (1952) and Mackiewicz (1972)] as established by Hunter (1930), are based chiefly on the: (1) position of the inner longitudinal parenchymal muscles with respect to the preovarian vitelline follicles [see Mackiewicz and Blair (1978), their Fig. 10], and (2) position of the gonopore within either the anterior or posterior half of the worm. Fuhrmann (1931) introduced, as a third criterion, the position of preovarian vitelline follicles. In the literature there is considerable disagreement as to the number of subfamilies, namely three (Yamaguti 1959, Bykhovskaya-Pavlovskaya et al. 1962, Schmidt 1970, Mackiewicz 1972), four (Hunter 1930, Wardle and McLeod 1952, Wardle, McLeod and Radinovsky 1974) or five (Fuhrmann 1931, Joyeux and Baer 1961).

Members of the subfamily Wenyoninae differ from the Caryophyllaeinae in having the gonopore in the anterior half of the worm. The subfamily Bovieninae supposedly differs from the Lytocestinae in having the preovarian vitellaria in two lateral bands. Mackiewicz (1963) has persuasively argued the unacceptability of gonopore position and vitellaria distribution as subfamily (family) characters and has advocated

the synonymy of the Wenyoninae and Bovieninae with the Caryophyllaeinae and Lytocestinae, respectively.

Johri (1959) felt that those caryophyllideans (such as Khawia) in which the preovarian vitellaria are cortical and postovarian vitellaria are both medullary and cortical, cannot be included in the Lytocestinae. He established the family Lallidae limited to those caryophyllideans with cortical preovarian vitellaria as well as with medullary post-ovarian vitellaria. Johri's Caryophyllaeidae included all worms having only medullary vitellaria; he divided them into two subfamilies (Wenyoninae and Caryophyllaeinae). Noting that many caryophyllideans lack postovarian vitellaria entirely, Mackiewicz (1963) rejected the distribution of postovarian vitellaria as a valid subfamily (family) character and rejected Lallidae as a valid concept.

Following discovery of a second species of Balanotaenia (B. newguinensis) and reexamination of B. bancrofti Johnston, 1924, Mackiewicz and Blair (1978) established a fourth family (Balanotaeniidae) of caryophyllideans. This family is characterized by cortical distribution of vitellaria and testes and by the medullary position of the ovary. Variation in placement of longitudinal parenchymal muscles has led some investigators (Szidat 1941, Janiszewska 1954, Mackiewicz and McCrae 1962) to question its use as a diagnostic character at the familial (subfamilial) level.

In this dissertation, caryophyllideans are considered as a distinct order, namely Caryophyllidea (class Cestoidea, subclass Eucestoda). I accept Mackiewicz and Blair's (1978) taxonomy and recognize four families: Caryophyllaeidae, Lytocestidae, Capingentidae and Balanotaeniidae.

Currently, those caryophyllideans in which the inner longitudinal muscles separate medullary testes from cortical vitellaria are assigned to the family Lytocestidae. According to Mackiewicz (1972), 13 genera are presently assigned to this family: Lytocestus Cohn, 1908; Caryophyllaeides Nybelin, 1922; Monobothrioides Fuhrmann and Baer, 1925; Djombangia Bovien, 1926; Lytocestoides Baylis, 1928; Bovienia Fuhrmann, 1931; Stocksia Woodland, 1937; Notolytocestus Johnston and Muirhead, 1950; Atractolytocestus Anthony, 1958; Lucknowia Gupta, 1961; Crecentovitus Murhar, 1963; Markevitschia Kulakovskaya, 1965 and Khawia Hsü, 1935.

Hsü (1935) erected the genus Khawia to include a new caryophyllidean (K. sinensis) of carp (Cyprinus carpio L.) from Peking, China. This species has a flat, fan-shaped scolex with frilled or wrinkled anterior margin ("scolex à bord anterieur fronce"). On the basis of the presence of cortical vitellaria, Hsü placed his genus in the Lytocestinae, and for a similar reason transferred Caryophyllaeus japonensis Yamaguti, 1934 from Caryophyllaeinae to this subfamily and considered C. japonensis as a species of Khawia.

Szidat (1937), unaware of the genus Khawia, proposed a new genus Bothrioscolex within the Lytocestinae and indicated B. japonensis as the genotype. He also added three more species to Bothrioscolex: B. prussicus, B. rossittensis and B. dubius, all recovered from Carassius carassius (L.) in East Prussia, Germany. Later in 1941, when he was aware of the genus Khawia, Szidat described K. baltica from Tinca tinca (L.) in East Prussia. Szidat (1941) preferred to retain the genus Bothrioscolex on the basis of the difference in the shape of its scolex from that of Khawia. Szidat (1937) described the scolex of Bothrioscolex as "vom Restkorper deutlich abgesetztem Scolex, dessen Stirnflache im fixierten Zustande spitz kegelformig gestaltet ist, im Leben aber einziehbar ist und dann eine tiefe Sauggrube bildet." This is in marked contrast to the frilled scolex of Khawia. Szidat (1941) also accepted B. japonensis as a valid species of Bothrioscolex because of the shape of its scolex. However, Yamaguti (1934) referred to C. japonensis as having "the anterior extremity with . . . crenulated anterior margin, truncate or conical according to the state of contraction . . . and . . . sharply constricted off from the other part of the body." This description is, in fact, characteristic of both Bothrioscolex and Khawia, Bothrioscolex being truncate and Khawia being crenulated. Szidat (1941) did indicate some doubts about separating the two genera on the

basis of such a variable scolex.

Wardle and McLeod (1952) did not mention Bothrioscolex and listed only K. sinensis and K. japonensis. In a review of European caryophyllideans (emphasizing the Polish fauna), Janiszewska (1954) followed Szidat in recognizing Khavia (sic) (Type species K. sinensis) and Bothrioscolex (Type species B. japonensis) as valid. She included among species of Bothrioscolex, B. rossittensis, B. prussicus and B. dubius.

Fotedar (1958) formally regarded Bothrioscolex as a synonym of Khawia and recognized six species, namely: K. sinensis, K. japonensis, K. prussicus, K. rossittensis, K. dubius and K. baltica. Yamaguti (1959) similarly synonymized Bothrioscolex with Khawia and listed K. sinensis, K. baltica, K. dubia (sic), K. japonensis and K. rossittensis as valid species.

In her review of the caryophyllidean fauna of the U.S.S.R., Kulakovskaya (1961) also suppressed Bothrioscolex; she recognized K. rossittensis, K. baltica and K. japonensis from waters of the Soviet Union. Following the suggestion of earlier Russian authors [including Bauer (1959)], Kulakovskaya also transferred two species of Caryophyllaeus, C. parvus Zmejew, 1936 and C. armeniacea Cholodkowski, 1915 to Khawia.

In their key to the parasites of freshwater fish of the U.S.S.R., Bykhovskaya-Pavlovskaya et al. (1962) added a sixth species of Khawia (K. sinensis) to the fauna of the

U.S.S.R. Kulakovskaya (1973), in the most recent review of caryophyllideans of the U.S.S.R., still listed only these same six species of Khawia.

Kulakovskaya and Krotas (1961) were the first to report K. sinensis from carp hatcheries of the western U.S.S.R. Until their report, it was believed that only one caryophyllidean, Caryophyllaeus fimbriceps Annenkova-Chlopina, 1919, parasitized carp in the Soviet Union. It was suggested that K. sinensis was introduced into the U.S.S.R. during the introduction of Amur carp from the Far East. Since 1961, K. sinensis has been extending its range westward and is apparently displacing C. fimbriceps as the predominant caryophyllidean of cultured carp (Bauer et al. 1969). There are recent reports of K. sinensis from Czechoslovakia (Přibyslavský et al. 1965), the Ukraine (Kulakovskaya and Ivassik 1967), Romania (Rădulescu and Georgescu 1966), East Germany (Mattheis and Spangenberg 1974), Poland (Pańczyk and Żelazny 1974) and Hungary (Murai and Molnár 1975).

Williams et al. (1980) provided a thorough description of K. armeniaca from Capoeta capoeta (Güldenstädt) and C. bushei Kessler from an Iranian river and reviewed the geographical distribution of this species. Paperna (1964) reported Kawia armenica (sic) from Barbus longiceps Cuvier and Valenciennes of the Sea of Galilee, Israel. Amin's (1978) record of Khawia sp. from Barbus bynni (Forsk.) of

the Nile River near Cairo, Egypt is the only report of this genus from Africa.

The first report of Khawia from the Nearctic was that the description of K. iowensis from carp in Iowa (Calentine and Ulmer 1961). In a key to the species of the genus, Calentine and Ulmer included K. sinensis, K. japonensis, K. prussicus, K. rossittensis, K. dubius, K. baltica and K. iowensis. However, Mackiewicz (1972) recognized only four species of Khawia: K. armeniaca, K. sinensis, K. japonensis and K. iowensis. The species K. parvus, B. prussicus and B. dubius were all placed as Incertae sedis because information was lacking on the disposition of longitudinal muscles. Mackiewicz made no mention of the additional species of Khawia or Bothrioscolex of other authors.

Several authors have also questioned the validity of certain species of Khawia. Janiszewska (1954) and Fotedar (1958) suggested that K. prussicus was a synonym of K. japonensis. Although he accepted the similarity between the two species on the basis of measurements and other characters, Szidat (1937) recognized B. prussicus and B. japonensis as unique because of their different hosts, separate geographical distribution and differing uterine coil lengths. Additionally, B. prussicus had been described by Szidat (1937) from a single specimen. Calentine and Ulmer (1961), however, felt that sufficient differences existed

between K. prussicus and K. japonensis to warrant retention as separate and distinct species.

Janiszewska (1954), too, questioned the validity of B. dubius. The specific title dubius indicated Szidat's uncertainty concerning its actual status. In her analysis of B. dubius, Janiszewska placed a question mark before the name, i.e., "?Bothrioscolex dubius." Szidat (1937) also labeled his figures 15 and 16 as "Bothrioscolex (?) dubius n. spec." Szidat placed this species in the genus Bothrioscolex with certain reservations. He had few specimens, made few observations on living worms and studied no sectioned material. The small size of Szidat's worms and the nature of their reproductive structures suggested to Janiszewska that B. dubius represented immature specimens of B. rossittensis.

Calentine and Ulmer (1961) described K. iowensis from carp of a number of lentic and lotic habitats throughout Iowa. A single immature specimen was also recovered from a bigmouth buffalo (Ictiobus cyprinellus Valenciennes). This parasite has since been reported from carp in California (Mackiewicz 1970, Hensley and Nahhas 1975), Oregon (Mackiewicz 1970), Oklahoma (Mackiewicz 1964, 1975), Kansas (Mackiewicz 1970), Nebraska (Williams 1980), North Dakota (Sutherland and Holloway 1979), Wisconsin (Anthony 1963, Williams 1977, 1980), Lake Erie (Ontario) (Dechtiar 1972), Tennessee

(Mackiewicz 1970) and North Carolina (unpublished data based on studies of specimens collected by M. R. Riggs, Biology Department, Wake Forest University, Winston-Salem, North Carolina). Williams and Sutherland (1981) have since found K. sinensis in carp from Oregon.

As stated previously, caryophyllideans utilize oligochaete annelids as intermediate hosts; however, members of the genus Archigetes Leukart, 1878 may become gravid while still within their annelid hosts. Pertinent literature on the progenetic development and life cycle of the genus Archigetes includes that by Wisniewski (1930), Kulakovskaya (1962b), Calentine (1963, 1964) and Kennedy (1965).

For caryophyllidean genera other than Archigetes, a two host life cycle (fish definitive host and oligochaete intermediate host) is characteristic. Although it has been generally accepted that fish become infected by eating oligochaetes harboring procercoids (Mackiewicz 1972), few experimental studies corroborating such an hypothesis exist. Only Mudry and Arai (1973a), working with Hunterella nodulosa Mackiewicz and McCrae, 1962, and Sapozhnikov (1972), working with K. sinensis, have experimentally infected laboratory maintained fish with procercoids and later recovered gravid worms. Studies dealing with procercoid development of Nearctic caryophyllideans include those by Calentine (1967), Calentine and Fredrickson (1965), Calentine and Williams (1967),

Calentine et al. (1970) and Williams (1978). It should be noted that Calentine (1967) was unable to rear procercoids of K. iowensis in any of four oligochaete species used in his study. Observations on procercoid development of Palearctic caryophyllideans were published by Sekutowicz (1934) and Kulakovskaya (1962a, 1964a).

Except for the recent description of the life cycle of K. japonensis by Demshin (1978), all other studies on procercoid biology and life cycles of Khawia concern K. sinensis (Pimenova 1971, Kupchinskaya 1972, Kotelnikov and Pimenova 1973, Sapozhnikov 1972, 1976, Demshin 1977). Tarakanov and Sapozhnikov (1973) have included reports of some success with cultivation of K. sinensis procercoids in artifical media.

Most species of caryophyllideans investigated thus far exhibit a seasonal periodicity in the definitive host. The most important studies dealing with the quantitative aspects of caryophyllidean seasonal periodicity in the fish host are those by Wunder (1939), Kanaev (1956), Kulakovskaya (1962c, 1964a,b), Calentine and Fredrickson (1965), Kennedy (1968, 1969), Lawrence (1970), Mudry and Arai (1973b), Anderson (1974, 1976), Williams (1979a,b) and Muzzall (1980a). Those by Kennedy and by Anderson are the most comprehensive, with particular attention given to an analysis of seasonal fluctuations in terms of host and oligochaete ecology and

population dynamics of C. laticeps in Leuciscus leuciscus L. and its intermediate host Psammoryctides barbatus (Grube, 1861). Those by Lawrence (1970) and Muzzall (1980a) are particularly noteworthy for their rigorous statistical analyses. Dubinina (1949), Rakova (1953), Bauer (1959), Kozicka (1959), Calentine and Ulmer (1961), Mackiewicz and McCrae (1962), Calentine (1962, 1964, 1967), Kulakovskaya et al. (1965), Mackiewicz (1965), Akhmetova (1966), Fredrickson and Ulmer (1967), Borgström and Halvorsen (1968), Sapozhnikov (1970, 1972), Williams and Ulmer (1971), Milbrink (1975), Grimes and Miller (1976) and Nakajima and Egusa (1978) present additional data on seasonal incidence. Mackiewicz (1972) published an excellent review and discussion of the literature dealing with caryophyllidean seasonal incidence.

Caryophyllideans are known to elicit considerable damage in fish (Mackiewicz et al. 1972) and oligochaete (Calentine et al. 1970) hosts. Studies on the effects of caryophyllideans on the vertebrate host are confined almost exclusively to Europe, particularly to the U.S.S.R., where fish farming involving carp, a common host for K. sinensis, is an important economic enterprise. Pertinent studies on the pathology elicited in carp by K. sinensis and control of the parasite through drug treatments of fish or by destruction

of oligochaetes include those by Musselius et al. (1963), Akhmetova (1966), Kulakovskaya and Svirepo (1967), Muzykovskiĭ et al. (1971), Sapozhnikov (1971, 1973), Pimenova (1971), Sapozhnikov and Vasil'kov (1972) and Vikhman and Kapoustina (1975). In North America, there has been considerably less attention given to pathology of caryophyllideans, largely because economically important fish are not normally involved.

It is apparent that K. sinensis is of considerable epizootic significance, especially in carp ponds of Europe and Asia. For this reason, considerable data on the life cycle, host-parasite ecology and pathology of this species have been compiled and appear in the literature. This dissertation will examine similar parameters for the closely related K. iowensis of wild carp in Iowa.

MATERIALS AND METHODS

Fish were collected with the use of backpack electrofishing and boat electrofishing equipment, fyke nets (modified hoop nets), minnow seines and variable mesh (experimental) gill nets. Type of habitat at each collecting site usually dictated the choice of specific collecting gear. Fish were immediately transported to the laboratory, maintained in aquaria containing filtered, continuously aerated lake water and necropsied within 24 hours of collection. Approximately 40 carp (20 each from a lentic and from a lotic habitat) were examined for the presence of helminths each month during the seasonal periodicity study. Fish were weighed, measured (standard and total length) and sexed; scale samples were taken from the left dorsolateral surface just below the dorsal fin and above the lateral line.

Location of helminths within the intestine was determined by dividing the gut into eight transverse sections of equal length, followed by longitudinal splitting of each gut segment and recording the number of worms of each species from each section. To facilitate visibility of helminths, each length of intestine was scraped with the blunt end of a forceps to remove mucus and other intestinal contents. Scraping the gut rarely dislodged attached worms from the mucosa. Individual sections of gut were examined against a

black background with the use of a 6X objective of a dissecting microscope. Gut contents and scrapings were diluted in fish saline (0.65% NaCl or $NaHCO_3$), agitated and examined with the use of a dissecting microscope.

Host and parasite data from each fish were coded, punched on computer cards and processed by computer. The following statistical tests were performed: Wilcoxon rank sum test, correlation and chi-square analyses, 2x2 contingency table and a modified t-test (analysis of independent samples when $\sigma_1 \neq \sigma_2$) (Snedecor and Cochran 1980). Values were considered statistically significant at $p < 0.05$. Incidence or prevalence is the percent of infected hosts in a given sample. Intensity is the number of worms per examined host. Mean intensity is the number of worms per infected host. The values following means are standard errors, unless otherwise stated.

Oligochaetes were collected by washing mud through a 36-mesh sieve. Annelids parasitized by larval caryophyllideans were identified by the conscpicuous appearance of whitish procercoids within the oligochaetes when the latter were viewed against a black background. Infected oligochaetes were placed in a small finger bowl containing lake water and examined with the use of a dissecting microscope. Because of its typically frilled scolex, Khawia, within its annelid

host, is easily distinguishable from procercoids of other North American caryophyllideans.

The excretory system of small living worms (less than one cm) was studied with the use of 43X and 97X objectives of a compound microscope. Nile blue sulphate and neutral red were added to some preparations, but details could best be studied with the use of distilled water alone. The excretory system of larger K. iowensis was studied by examining sectioned material.

Gravid and mature Khawia were placed in distilled water for an hour prior to fixation in warm ten percent buffered formalin; such worms increased considerably in length. Calentine and Ulmer (1961) noted that stained specimens of K. iowensis prepared in this way showed internal structural details much more clearly than did worms fixed immediately upon removal from the host. Similar observations were made during the present study.

Immature cestodes from fish and procercoids from annelids were fixed in hot AFA. Oligochaetes were preserved in cold ten percent buffered formalin. Acanthocephalans were refrigerated in distilled water for several hours and then fixed in cold AFA. Nematodes were fixed in hot glycerine alcohol.

Wholemounts of cestodes and acanthocephalans were stained in either Mayer's paracarmine or Semichon's acetocarmine with fast green counterstain, and were cleared in

methyl salicylate. Nematodes were cleared in glycerin and mounted on slides using the double coverslip method (Cable 1958). Material to be sectioned was dehydrated in tertiary butyl alcohol, embedded in paraffin and sectioned at eight µm as prescribed in Humason (1967) and Luna (1968). Sections were stained in Harris' hematoxylin with eosin counterstain. After dehydration in ethanol, sections were cleared in xylene. Resinous mounting media were used for all preparations of cestodes and acanthocephalans. Drawings were made with the use of a microprojector or camera lucida.

The length and maturity of approximately 98% of the Khawia recovered from the Little Sioux River were determined. Designations of maturity were as follows: (1) gravid worms, containing intrauterine eggs, (2) mature, nongravid worms, possessing all genital structures but no eggs, and (3) immature worms, lacking development of one or more genital structures.

Gravid specimens of Khawia were stimulated to shed eggs by refrigerating parasites in distilled water for several hours. Additional eggs were often obtained by tearing apart the bodies of gravid cestodes. Eggs were washed with filtered lake water to remove debris and then maintained in 400 ml beakers at room temperature (18-22 C). Filtered lake water was used in all egg cultures and culture water was changed twice weekly. For purposes of study, eggs

were transferred to a glass slide, covered with a coverslip and examined with the use of a compound microscope.

Exposure of embryonated eggs to oligochaetes involved use of a mud medium. Mud used in all experimental studies was obtained from streams, screened through a 36-mesh sieve and autoclaved for 45 minutes at a pressure of 15 psi. Approximately 500 embryonated eggs of K. iowensis from carp were added to about 20 cc of sterilized mud in a 200 ml beaker. Approximately 100 oligochaetes were added and maintained under continuous aeration at room temperature. Following exposure to eggs for 24 hours, annelids were removed and maintained at 18-22 C in aerated two-liter aquaria containing mud. Occasionally, a second group of annelids was added to the container following removal of the first batch of worms. Oligochaetes used in all experimental studies were either laboratory reared specimens or worms collected from nature and maintained in the laboratory three to nine months prior to use. All oligochaetes were examined for the presence of procercoids prior to use in feeding experiments. All oligochaetes were fed periodically (usually once per month) a blended mixture of cornmeal, oatmeal and commercial flaked fish food.

For the first three days following exposure to eggs, oligochaetes were examined daily to observe oncosphere

penetration and migration and procercoid development. Thereafter, oligochaetes were examined at three day intervals for the presence of developing procercoids. During the first month post-exposure, this was done by placing oligochaetes on a glass slide in a drop of spring water, covering them with a coverslip and examining at a magnification of 100 diameters with a compound microscope. After about 30 days, larvae had grown sufficiently within the oligochaete to be readily discernible with the use of a dissecting microscope.

Because laboratory reared carp were not available for feeding experiments, specimens were taken from nature. However, such carp were obtained in late November when incidence and intensity of infection with Khawia were known to be at low levels. Only carp five cm or less in total length were retained and reared in the laboratory. Previous examination of similarly sized carp had shown such small fish to be only rarely infected. Immediately following capture, approximately fifty of the small carp were examined for the presence of Khawia; no infections were observed. As a final check prior to initiating feeding studies, feces of all fish were examined for the presence of cestode eggs; no eggs were seen.

Goldfish, Carassius auratus (L.), used in a limited number of feeding experiments were obtained from commercial sources. On one occasion, however, large goldfish from Lake Laverne (located on the Iowa State University campus) were used for a

specific experiment. Examination of fifty of these goldfish prior to conducting experimental feedings revealed no caryophyllideans in the population.

Small carp and goldfish were reared in the laboratory for one to two years prior to experimental feedings. Fish were maintained in 200-liter fiberglass aquaria. Both a static system using artificial spring water (Ulmer 1970) and a flow-through system using filtered lake water were employed. All fish were maintained at 16-21 C and fed a diet composed of frozen brine shrimp and commercial flaked fish food. Carp were reared to lengths varying between 15 and 20 cm.

In feeding experiments involving K. iowensis and small fish (five to 15 cm), the latter were allowed to ingest infected oligochaetes. Fish were placed in individual, clean two-liter aquaria. Several infected annelids were then introduced into each aquarium. Fish were allowed to eat the worms, and bottom contents of aquaria were examined periodically for the presence of regurgitated cestodes. Fish exposed to tapeworms were given frozen brine shrimp and commercial fish food ad libitum and were examined at various intervals following exposure.

Larger fish (greater than 15 cm) were force fed with the use of an intubation device patterned after that of Loeb and Kelly (1960). This apparatus consisted of a 20 cm length of aluminum tubing (seven mm in diameter), equipped at one end

with a large rubber bulb. The tube was bent slightly to conform to the curvature of the pharyngeal region of the carp. Infected oligochaetes were placed in a gelatin capsule or were confined between pieces of cotton at the end of the tube. The tube was introduced into the stomach of the fish and the contents were discharged by compressing the rubber bulb. Large fish exposed to cestodes were maintained for one day in individual 30-liter aquaria in order to monitor possible regurgitation of worms. Fish were then transferred to 200-liter aquaria, maintained on an *ad libitum* diet of brine shrimp and examined at various intervals following exposure.

SUMMARY OF LIFE CYCLE

Gravid specimens of Khawia iowensis occur within the intestinal tract of the cyprinid fish, Cyprinus carpio. Examination of cyprinids and catostomids native to Iowa, as well as introduced carp and goldfish, reveals diverse caryophyllidean faunas limited to catostomids and carp. Except for the report by Calentine and Ulmer (1961) of accidental infection in a bigmouth buffalo (Ictiobus cyprinellus) with an immature Khawia, K. iowensis has been found only in carp. Carp infections are strictly seasonal, with maximum incidence and mean intensity occurring in August. Sexually mature carp may harbor more than 800 K. iowensis, but immature carp normally harbor less than 30.

Eggs shed by parasites are unembryonated and pass from the fish in the host's feces. Oncosphere development occurs in the bottom sediments and requires about 17 days. Under experimental conditions, Aulodrilus pigueti becomes infected by ingesting embryonated eggs. Exposure of embryonated eggs to five additional species of oligochaetes did not result in procercoid development. After hatching in the tubificid's intestine, oncospheres penetrate the gut wall in the posterior part of the intestinal tract and migrate anteriad. Procercoid development occurs in the anterior portion of the

annelid's coelom. A cercomere bearing six characteristic embryonic hooks appears at approximately 40 days. By 50-60 days, the fimbriated scolex is well-developed. Infective procercoids of K. iowensis are not progenetic; the only gonadal anlage is a small, hourglass shaped accumulation of cells in the region of the incipient ovary.

Fish apparently acquire infections of K. iowensis by ingestion of infected oligochaetes. Factors responsible for the periodicity of adults in carp are not thoroughly understood. However, evidence suggests that among carp this periodicity may result from seasonal variations in: (1) feeding activities, (2) hormonal levels and/or (3) immune responses.

EGGS AND ONCOSPHERES

Gravid K. iowensis, having been placed in distilled water for one hour, normally shed several hundred eggs. Eggs from both sources are unembryonated and average 57 by 46 µm in size. Viable eggs are light brown and the egg shell is covered with minute spines approximately three µm in length. Sections of gravid worms were examined to determine, if possible, whether eggs are spinose from the time of their formation in the ootype or whether spines develop during passage through the uterus or after their release from the adult cestode. However, no spines were evident in sections of intrauterine eggs.

As is typical of caryophyllideans, the egg is operculate and bears a minute boss at its posterior end. Margins of the operculum are difficult to distinguish. Crushing eggs through coverslip pressure usually results in a longitudinal splitting of the shell but occasionally a few eggs will pop open at the operculum. Eggs freshly removed from a gravid worm contain five to nine yolk cells, measuring five through seven µm in diameter; such cells constitute most of the volume of the egg. Additional small granules of

undetermined nature are scattered amongst the yolk cells. A zygote, measuring approximately 12-13 μm in diameter, is visible in sections of intrauterine eggs. There is no further development of the egg until it reaches water.

After five days in lake water, an irreguarly shaped embryo becomes distinctly visible; no internal differentiation is evident in fresh preparations. By the tenth day, embryos have elongated and measure 35 μm in length. Between the tenth and fifteenth day, three pair of embryonic hooks appear and the oncosphere increases in size. In all eggs examined, hooks are directed toward the anopercular end. The medial pair of hooks is slightly longer (12 μm) than either of the two lateral pairs (ten μm). Flame cells were not identified in living oncospheres. In contrast to the ciliated coracidia of pseudophyllideans, no cilia were observed on K. iowensis oncospheres at any time during their development.

At about day 15, two large, granular cells become evident near the medial pair of hooks. Similar cells have been described or illustrated for eggs of most caryophyllideans in which embryonation has been followed. Wisniewski (1930) expressed belief that such cells are associated with production of embryonic hooks, whereas Mudry and Arai (1973a) suggested they may possibly be penetration glands. Demshin

(1978) noted a distinct tubular connection between these cells and the medial pair of hooks during penetration of the tubificid gut by K. japonensis oncospheres.

The average time of oncosphere development for K. iowensis is between 16 and 18 days. The oval oncosphere usually lies along the long axis of the egg and measures 44 by 25 µm. In the laboratory, living, unhatched oncospheres of K. iowensis may be maintained for three months or more at room temperature. Eggs maintained under refrigeration (4 C) do not embryonate. Oncospheres within eggs exhibited little activity other than occasional movement of embryonic hooks and limited contraction and elongation of the body. Hatching of eggs was never observed and no eggs with opened opercula were ever seen in laboratory cultures.

Embryonation of K. iowensis eggs in the present study was nearly identical to that observed by Calentine and Ulmer (1961) (see their Figs. 11-17). Only slight variations in the measurements of developing eggs and oncospheres were noted between the two studies. The slower rate of development observed in the more recent study (16-18 days versus 15 days) may be explained by the cooler temperatures at which eggs were incubated. In the present study, egg incubation experiments were conducted in the air conditioned wet lab in King Laboratory at Iowa Lakeside Laboratory.

Bauer et al. (1969) noted that eggs of K. sinensis meas-

ured 42-48 by 25-30 µm. Kulakovskaya (1962a) found that K. sinensis eggs embryonated in 30-32 days under laboratory conditions (no temperature given). Oncospheres of K. sinensis lived for three months. In studying the life cycle of K. japonensis, Demshin (1978) found that eggs measured 56-62 by 42-50 µm. At 18-20 C, well-formed oncospheres of K. japonensis were evident within 12-14 days of incubation but were not infective until days 30-31. Egg size appears to be one consistent character by which K. sinensis may be separated from K. iowensis and K. japonensis.

INTERMEDIATE HOSTS

General Account

Larval caryophyllideans have never been recorded from aquatic invertebrates other than oligochaetes (Mackiewicz 1972). Khawia sinensis may develop in a wide variety of oligochaete hosts. Kulakovskaya (1962a) observed the development of several caryophyllideans under both experimental conditions and natural conditions of the Ukraine. She recorded Limnodrilus udekemianus Claparède, 1862, L. hoffmeisteri Claparède, 1862, Tubifex tubifex (Müller, 1774) and Potamothrix hammoniensis (Michaelsen, 1901) as suitable intermediate hosts for K. sinensis. In the Lvov region, Kulakovskaya et al. (1965) found not only the above mentioned tubificids as intermediate hosts for K. sinensis but also recorded P. barbatus as an oligochaete host. Sapozhnikov (1972) found Ilyodrilus templetoni (Southern, 1909) as well as T. tubifex, L. udekemianus, L. hoffmeisteri and P. hammoniensis to be intermediate hosts of K. sinensis in carp ponds of the Moscow region. In a later study (1976), she did not find L. claparedeianus Rattzel, 1868, L. profundicola (Verrill, 1871) or Psammoryctides albicola (Michaelsen, 1901) of carp ponds in the Moscow region to be infected with K. sinensis. In the Primorsk Territory, Demshin (1977) reported natural infections of K. sinensis from L. udekemianus and Limnodrilus sp.

Under experimental conditions, Demshin (1978) found L. udekemianus to serve as the intermediate host of K. japonensis. The life cycles of other Palearctic species of Khawia have not been investigated.

Calentine (1967) was unable to infect L. hoffmeisteri, I. templetoni, Branchiura sowerbyi Beddard, 1892 and Dero limosa Leidy, 1852 = Dero digitata (Müller, 1773) with embryonated eggs of K. iowensis. Calentine's study was conducted using oligochaetes and cestodes collected from Iowa. Dennis Williams (Plant Pathology, Seed and Weed Science Department, Iowa State University, Ames, Iowa, personal communication) attempted unsuccessfully to infect a number of species of Wisconsin oligochaetes with embryonated eggs of K. iowensis originally obtained from Wisconsin carp. Recently, R. Calentine (Biology Department, University of Wisconsin, River Falls, Wisc., personal communication) noted the hatching of K. iowensis eggs in the gut of T. tubifex. Oncospheres successfully penetrated the gut wall; but within 24 hours, Calentine observed all larvae to be dead.

Six oligochaete species, namely Aulodrilus pigueti Kowalewski, 1914, L. hoffmeisteri, L. udekemianus, I. templetoni, D. digitata and Stylaria lacustris (L., 1767) were recovered from the Little Sioux River during the course of this investigation. Limnodrilus hoffmeisteri, I. templetoni and A. pigueti are known to predominate in shallow muddy

areas of negligible current, whereas L. udekemianus prefers riverine areas with a more rapid current and sandy bottom (M. Loden, Jefferson Parish Environmental and Development Control Department, Jefferson Parish, Louisiana, personal communication). Because of the sluggish flow of the Little Sioux River and the absence of riffle areas, L. udekemianus was rarely encountered. The two naidid species (D. digitata and S. lacustris) were restricted to shallow, slow moving waters where emergent vegetation was present.

Caryophyllidean procercoids were found in a number of oligochaetes from the Little Sioux River, but even after examination of several thousand oligochaetes, larval K. iowensis were not found. Although L. hoffmeisteri, I. templetoni, D. digitata, S. lacustris and A. pigueti were later exposed to embryonated eggs of K. iowensis under experimental conditions, development occurred only in A. pigueti. Feeding experiments with L. udekemianus were not conducted because of the limited number of these hosts available for experimental study. The role of this oligochaete as a possible host for K. iowensis thus could not be ascertained. However, the relative scarcity of L. udekemianus in the Little Sioux River indicates that its role in nature would seem to be negligible.

Failure to find natural infections indicates that infection rates of oligochaetes with K. iowensis are extremely

low. Mackiewicz (1972) noted that in natural habitats, incidence with caryophyllidean larvae is invariably low, usually less than three percent and rarely above 15%, but under fish-farming conditions it may be as high as 50%. Calentine (Biology Department, University of Wisconsin, River Falls, Wisc., personal communication) and Williams (Plant Pathology, Seed and Weed science, Iowa State University, Ames, Iowa, personal communication) have extensively examined the oligochaete faunas of West Central Wisconsin where carp are frequently infected with K. iowensis. Neither investigator has ever recovered an oligochaete infected with Khawia.

Other common invertebrates of the Little Sioux River were also examined for the presence of caryophyllidean larvae. None of several hundred midge larvae (Chironomidae) or burrowing mayfly larvae (Hexagenia sp. Walsh, 1863) were infected with caryophyllideans, however.

Experimental Studies

Experimental studies on K. iowensis were conducted with eggs derived from two sources: those shed from gravid worms maintained in distilled water and those dissected from gravid worms. Eggs were maintained at room temperature in lake water for at least 30 days prior to use in experiments. Feeding experiments were conducted by mixing embryonated eggs with a small quantity of mud, to which oligochaetes were then added.

The oligochaetes, B. sowerbyi, L. hoffmeisteri, I. templetoni, A. pigueti, D. digitata and S. lacustris were exposed to embryonated eggs of K. iowensis. Infections resulted, however, only in A. pigueti (Table 1).

Table 1. Summary of feeding experiments conducted with oligochaetes and embryonated eggs of Khawia iowensis. Incidence and mean intensity are based on infections at day 40 post-feeding

Species	Number exposed	Egg incubation time (days)	Number infected (%)	Number multiple infections (%)	Max. No.
Aulodrilus pigueti	181	32	27 (15)	10 (6)	7
Limnodrilus hoffmeisteri	102	32	0		
Ilyodrilus templetoni	97	33	0		
Branchiura sowerbyi	78	34	0		
Dero digitata	104	37	0		
Stylaria lacustris	100	37	0		

Dero digitata and S. lacustris, both small species of the family Naididae, average between five and ten mm in length and may be too small to ingest eggs of K. iowensis. Calentine et al. (1970) found that only Glaridacris confusa Hunter, 1929 would begin development in these two oligochaete species. The eggs of G. confusa (measuring 37-48 by 20-31 µm) are considerably smaller than the eggs of K. iowensis or eggs of the six other caryophyllideans studied by Calentine's group.

That the tubificid species used in the present study are capable of ingesting K. iowensis eggs has been demonstrated by Calentine et al. (1970). In their experiments, eggs of G. catostomi hatched and began development in B. sowerbyi, L. hoffmeisteri, I. templetoni and A. pigueti. Eggs of G. catostomi (averaging 60 by 42 μm) are larger than K. iowensis eggs.

During the experimental studies, A. pigueti of all sizes became infected with K. iowensis. This is in decided contrast to the results of a number of investigations (Calentine 1963, Kupchinskaya 1972) in which the smaller sexually immature tubificids were much more likely to become infected with caryophyllidean cestodes than the larger sexually mature tubificids. This incongruity may be related to the small size of A. pigueti with respect to most other adult tubificids.

Although hatching of the oncosphere and its penetration of the oligochaete's intestinal tract were not observed, oncospheres were found within the annelid coelom within three hours of exposure to eggs. Penetration occurred between segments XX and XXXII. At five days post-feeding, larvae located between segments XIV and XX. At 25 days, all larvae were located between segments X and XVIII. During their anteriad migration, frequent movement of the embryo occurred especially in the region of the developing scolex; during

such movement, the hooks are directed posteriorly. Developing procercoids were never attached to the coelomic wall.

During the first 25 days within tubificids, procercoids are oval in outline. At day 15, the developing procercoid (still lacking the cercomere) measures approximately 0.2-0.3 mm in length (Fig. 1). By day 25, procercoids measure 0.48-0.55 by 0.11-0.20 mm. Constriction of the posterior portion of the body results in formation of the cercomere by day 40 (Fig. 2); the embryonic hooks are present on the caudal end of the cercomere. At this time, the entire procercoid measures 0.79-0.88 by 0.29-0.40 mm. The shape of the scolex is usually truncate but may also be arrowhead-like. Anlage of the reproductive organs are also evident in the region of the incipient ovary. By day 60, the procercoid has developed a scolex typical of adult K. iowensis and the reproductive anlage is in the shape of an hour-glass (Fig. 3). Analysis of thirty 60 day old procercoids revealed that the body (without the cercomere) averages 1.06 in length (range 0.65 to 1.40 mm) and that the cercomere measures approximately 0.31 in length (range 0.15 to 0.45 mm). Infected A. pigueti were examined up to 100 days post-infection, but further morphological development was not observed.

The location of developing procercoids seems to vary among caryophyllidean species. In the genus Archigetes, for example, procercoids have been described from the coelem, seminal vesicles and ovisacs (Calentine 1962). Kulakovskaya (1962a)

described C. laticeps and K. sinensis as developing within the coelom of the tubificid hosts. Mudry and Arai (1973a) found that procercoids of H. nodulosa develop in the seminal vesicles of L. udekemianus.

Procercoids of K. iowensis develop within the coelom of the intermediate host, A. pigueti. Procercoids are not found within gonads. Species of this oligochaete, however, reproduce asexually and well-developed reproductive organs have not been described (Brinkhurst and Jamieson 1971). Degenerate testes often appear in segment V as well as in segment VI of the maturing tubificid, but no associated male ducts have been described. Additionally, in most accounts, a mass of spongy tissue is described as filling a crescent-shaped area of segment VI, this mass being thickest ventrally, and extending into segments V and VII in some specimens. The function of this tissue is unknown. Commonly, the rudiments of reproductive organs are located more anteriorly than among tubificids in general.

Most of the 117 K. iowensis procercoids analyzed, eventually localized in segments VIII through XIII (range VI through XV). In single infections, cestodes are usually fully extended, with scoleces directed toward the anterior or posterior of the host's body. Parasites in multiply infected hosts may be doubled upon themselves. Procercoids usually extend over a distance of three to four segments (range one to seven, average 3.55).

Development of K. iowensis procercoids very closely corresponds to that seen for K. sinensis and K. japonensis by Kulakovskaya (1962a) and Demshin (1978), respectively. For these three species of Khawia, procercoid development does not proceed beyond Kennedy's (1965) "Stage II," meaning that only cercomere and genital rudiments are present. This is in marked contrast to Archigetes and Caryophyllaeus where progenetic procercoids develop (Stage III); in Archigetes, even egg production (Stage IV) may result (Mackiewicz 1972). Procercoids of Khawia spp. are thus infective to carp at an early stage of development. Kulakovskaya (1962a) states that carp may acquire K. sinensis infections even before formation of the scolex.

As far as procercoid development is concerned, few significant differences exist among the three Khawia species. Only minor variations in rates of procercoid development and sizes of corresponding larval stages are evident. The most marked difference in procercoid development for the three Khawia species concerns tubificid host specificity. According to Sapozhnikov (1976), a wide variety of tubificid genera and species is known to serve as suitable intermediate hosts for K. sinensis; K. japonensis and K. iowensis, on the other hand, appear to be monospecific with respect to suitable tubificid hosts. Demshin (1978) found only L. udekemianus

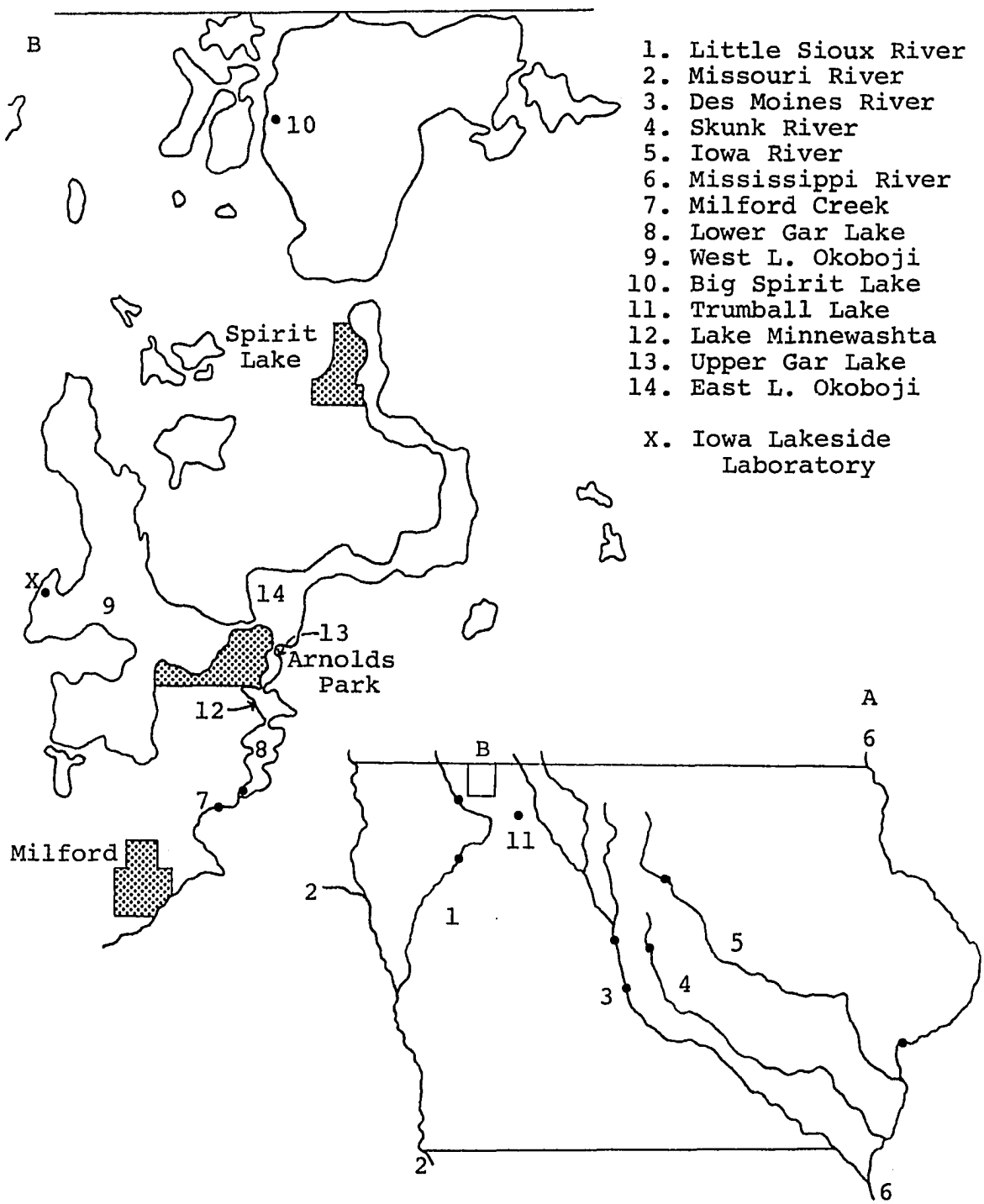
to serve as a host for K. japonensis, and, in the present study, only A. pigueti became infected with K. iowensis. Distributions of all known tubificid hosts for Khawia spp. are cosmopolitan (Brinkhurst and Jamieson 1971).

DEFINITIVE HOSTS

Geographical Distribution

It was previously noted (see Introduction) that K. iowensis has been reported widely in North America, from California to North Carolina. In the present study, carp from five rivers (Little Sioux, Des Moines, Iowa, Skunk, Mississippi) in Iowa and from four Iowa lakes (West Okoboji, Big Spirit, Lower Gar, Trumbull) were examined for the presence of Khawia and other intestinal helminths (Map 1). Except for the single carp collected from the Mississippi River, K. iowensis parasitized carp in all these areas. Archigetes iowensis Calentine, 1962 was recovered only from C. carpio of the Iowa River. A third caryophyllidean of carp, Atractolytocestus huronensis Anthony, 1958, is reported from Iowa for the first time (Lower Gar and West Okoboji lakes and Little Sioux and Mississippi rivers).

West Okoboji, Big Spirit, Lower Gar and Trumbull lakes and the Little Sioux River are within the Missouri River drainage system and the Des Moines, Skunk and Iowa rivers are all associated with the Mississippi River drainage. Since these two systems drain all surface waters of the state and since they offer similar ecological habitats with suitable tubificid hosts, it seems likely that K. iowensis occurs throughout Iowa wherever carp are found.



Map 1. Location of collections within Iowa (A). Dots indicate collection sites. "Iowa Great Lakes" study area (B) is enclosed with solid lines

Infection Parameters

Nine species of catostomids, six species of cyprinids and 18 additional species of fish belonging to other families were examined for intestinal helminths during this investigation. A total of 1,362 fish was examined. Khawia iowensis was recovered only from the cyprinid, C. carpio. Fish examined and percentage of infection with K. iowensis and other caryophyllideans are listed in Table 2. Caryophyllideans were found only in catostomids and C. carpio.

Carp collections from the Little Sioux River were made in 1979 during each month beginning in April and continuing through November and, in 1980, monthly from March through November. Data available for the winters (December through February) of 1979-80 and 1980-81 are based on single collections made in January 1980 and February 1981, respectively. Collecting during the winter months is extremely difficult because of heavy ice cover, low river depth and tendency of carp to school throughout winter months (Johnson and Hasler 1977).

In 1979, carp collections from Lower Gar Lake were made during the months of May through October. Because of the extremely shallow nature of the lake (maximum depth of 1.5 meters), it is likely that carp in winter migrate from Lower Gar Lake, into Lake Minnewashta, then into Upper

Table 2. Infection of Iowa cyprinid and catostomid fish with caryophyllideans during 1977-81. Sites at which caryophyllideans were recovered are numbered according to legend of Map 1 (p. 42)

Fish Caryophyllidean	No. exam.	No. inf. (%)	Mean intensity	Site
Cyprinidae				
Cyprinus carpio	873			
Khawia iowensis		527(60)	14.1	1,3,4,5,8,9, 10,11
Atractolytocestus huronensis		44(5)	27.6	1,6,8,9
Archigetes iowensis		1(1)	4.0	5
Carassius auratus	41	0		
Semotilus atromaculatus	22	0		
Notropis cornutus	10	0		
Notropis lutrensis	11	0		
Notemigonus crysoleucus	4	0		
Pimephales promelas	2	0		
Catostomidae				
Ictiobus cyprinellus	63			
Glaridacris laruei		48(76)	17.7	8,9
Monobothrium ingens		27(43)	2.3	8,9
Ictiobus bubalus	5			
Glaridacris laruei		1(20)	4.0	3
Catostomus commersoni	118			
Glaridacris catostomi		27(23)	9.1	1,8
Glaridacris oligorchis		13(11)	32.4	1,8
Biacetabulum biloculoides		20(17)	4.6	1,8
Biacetabulum macrocephalum		14(12)	3.6	1,8
Hunterella nodulosa		12(10)	2.3	1,4,8
Monobothrium hunteri		11(9)	3.9	1
Carpiodes carpio	81			
Spartoides wardi		51(63)	19.1	1,3,4,6,8
Biacetabulum carpiodi		14(17)	2.0	1,6
Carpiodes cyprinus	5			
Spartoides wardi		3(60)	6.7	3,4
Moxostoma macrolepidotum	21			
Isoglaridacris longus		7(33)	6.0	3
Glaridacris oligorchis		2(9)	1.5	1,3
Moxostoma erythrurum	8			
Isoglaridacris folius		2(25)	2.0	3
Hypentelium nigricans	5			
Monobothrium ulmeri		2(40)	2.5	3

Gar Lake and finally into the deeper East Lake Okoboji where they overwinter (Map 1) (personal communication, Wally Jorgensen, Iowa Conservation Commission, Orleans, Iowa). Despite intensive fishing using electrofishers, seines, gill nets and fyke nets in November 1979 no carp were collected from Lower Gar Lake which normally is ice covered from late November through early April.

The intestinal helminth fauna of Little Sioux River and Lower Gar Lake carp consisted of K. iowensis, A. huronensis, Glaridacris oligorchis Haderlie, 1953, Pomphorhynchus bulbocolli (Linkins, 1919), Leptorhynchoides thecatus (Linton, 1891), Neoechinorhynchus sp. Hamann, 1892, Corynosoma sp. Lühe, 1904, Capillaria sp. Zeder, 1800 and Camallanus sp. Railliet and Henry, 1915. Except for K. iowensis, A. huronensis, P. bulbocolli and Capillaria sp., all other helminths recovered from carp probably represent accidental infections, since they were only rarely encountered in carp of either habitat and since parasite specimens were always immature. However, L. thecatus was a dominant helminth in percid and centrarchid fish of Lower Gar Lake; Camallanus ancylodirus Ward and Magath, 1916 commonly infected Lower Gar Lake I. cyprinellus, and C. oxycephalus Ward and Magath, 1916 was found in a wide variety of Lower Gar Lake fish. Accidental infections of carp from the Little Sioux River may be explained by the high incidence and intensity in the

river of G. oligorchis in Catostomus commersoni (Lacépède), N. prolixus Van Cleave and Timmons, 1952 in Carpiodes carpio (Rafinesque) and C. constrictum Van Cleave, 1918 in a variety of nesting waterfowl.

Of the four predominant helminths (K. iowensis, A. huronensis, P. bulbocolli and Capillaria sp.), only P. bulbocolli was recovered from a fish species other than carp. Gravid females of this acanthocephalan occurred as well in I. cyprinellus, Carpiodes carpio, C. commersoni, Ictalurus melas (Rafinesque) and Aplodinotus grunniens (Rafinesque).

During the study, K. iowensis was the most frequently encountered helminth. The large infrapopulations of K. iowensis emphasize the large numbers of oligochaetes ingested by carp. Few studies of food habits of carp and catostomids show that oligochaetes are common food items of these bottom feeding fish (Rehder 1959, Summerfelt et al. 1970, Trafanelli et al. 1970). Summerfelt et al. (1970) incongruously state that no oligochaetes were found in intestinal analyses of carp from Oklahoma, that oligochaetes were abundant in the macrobenthos of the reservoirs examined, and that carp apparently feed on the unconsolidated (flocculent) portion of the substrate. According to Scott and Crossman (1973), carp will suck up a mouthful of bottom ooze and detritus, expel it into the water and select food items.

It has been my experience that carp do, in fact, ingest large numbers of oligochaetes and that the fragile body wall of these annelids is rapidly digested. In the present study, fish collected by use of electrofishers were more likely to contain oligochaetes or oligochaete parts in their intestinal tracts than were fish collected in fyke or gill nets. Earlier food habit studies were often based on such net-collected fish obtained from commercial fishermen.

The incidence (2x2 contingency table, $p < 0.001$), and mean intensity (modified t-test, $p < 0.001$) of K. iowensis were higher in Little Sioux River than in Lower Gar Lake hosts (Table 3). The lower prevalence in Lower Gar Lake carp (48 vs. 70%) and lower mean intensity (6.0 vs. 16.7 worms per infected host) may be attributed to three main factors. Firstly, the intermediate host of K. iowensis, A. pigueti was found only rarely in mud collections from Lower Gar Lake, but was one of the predominant oligochaetes in the Little Sioux River. Even though Lower Gar Lake has a bottom consisting primarily of fine mud and ooze and supports a diverse oligochaete fauna, suitable intermediate hosts are insufficient to support a large suprapopulation of K. iowensis. Secondly, since carp migrate from Lower Gar Lake during winter months, there is a considerable period of time during which cestode eggs are not available to oligochaetes. Thirdly, there may be

Table 3. Prevalence and mean intensity of the predominant helminth species recovered from 522 Little Sioux River carp and 255 Lower Gar Lake carp

	Prevalence	Mean intensity ± SD	Intensity range	Total number recovered
Little Sioux River				
K. iowensis	70	16.7 ± 2.6	0-815	6,075
A. huronensis	1	7.0 ± 2.1	0-10	28
P. bulbocolli	32	5.1 ± 0.7	0-64	839
Capillaria sp.	20	7.8 ± 1.0	0-65	832
Lower Gar Lake				
K. iowensis	48	6.0 ± 0.8	0-67	738
A. huronensis	14	5.2 ± 3.3	0-116	181
P. bulbocolli	68	12.2 ± 2.7	0-403	2,119
Capillaria sp.	20	80.2 ± 29.3	0-1,338	4,171

differences in the feeding activities of carp from rivers and those from lakes [see Nikolsky (1963].

Whereas P. bulbocolli infected Lower Gar Lake carp more often than Little Sioux River carp (2x2 contingency table, $p < 0.001$), the mean intensities of both P. bulbocolli and Capillaria sp. were significantly higher (modified t-test, $p < 0.05$). in Lower Gar Lake than in Little Sioux River hosts (Table 3). The greater worm burdens of P. bulbocolli in Lower Gar Lake carp may be explained by two factors. The amphipod intermediate hosts for Pomphorhynchus were only infrequently collected from the Little Sioux River but were a major component of the littoral fauna of Lower Gar Lake. Also, other suitable definitive hosts for P. bulbocolli, including I. cyprinellus, I. melas and C. commersoni, had large, well-established populations in Lower Gar Lake but were infrequently collected in the riverine habitat. With more suitable definitive and intermediate hosts in the lentic habitat, the larger suprapopulations of P. bulbocolli in Lower Gar Lake would be expected. No reasons can presently be offered to explain the differences in infection levels of Capillaria between Lower Gar Lake and Little Sioux River hosts.

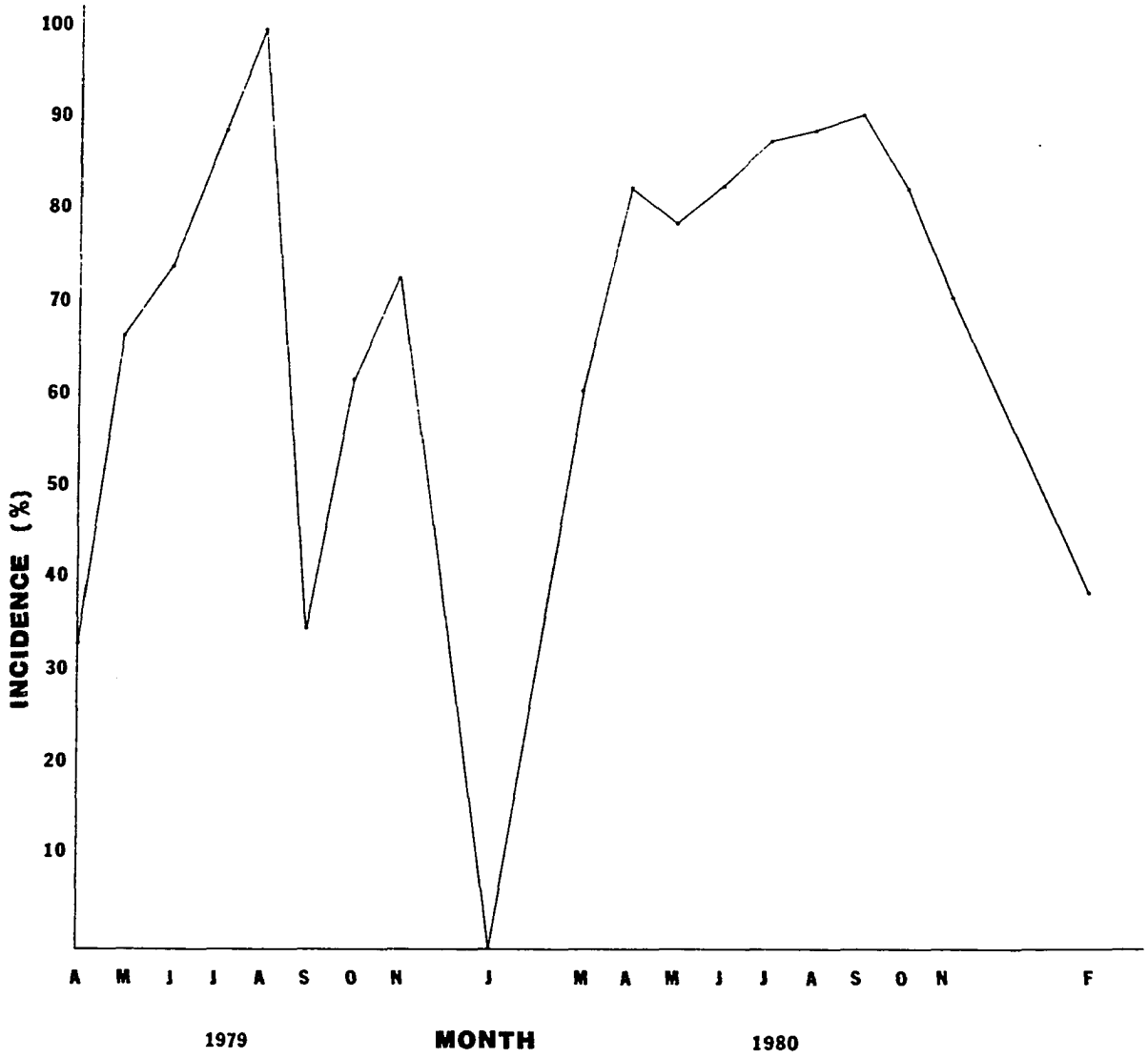
Seasonal Infection Patterns

One of the striking characteristics of K. iowensis is its seasonal distribution in carp; this periodicity occurred during both years (1979-80 and 1980-81) of field studies

(Graphs 1 and 2, Table 4). Prevalence and mean intensity were highest in summer 1979 and 1980. Significant differences (2x2 contingency table, $p < 0.05$) exist between summer seasonal prevalences when contrasted to the other seasons. The summer mean intensities are also higher (Wilcoxon rank sum test, $p < 0.05$) than at other seasons. During winter and early spring months, percentage of infection in carp is at its lowest mark. The January 1980 collection is composed entirely of carp less than 25 cm in total length and does not represent the wider variety in sizes of fish collected at other times of the year.

Khawia iowensis also exhibited seasonal patterns in maturation (Graph 2), but there is little evidence of any seasonal variation in length distribution of three maturation classes (Graph 3). Egg production occurs from late spring through late fall. Except for the abnormal collection for winter 1980, cestodes of all sizes (1.2-24.9 mm) were found during winter and early spring. No gravid worms were recovered during winter collections. Of worms collected in March 1980 and in February 1981, only relatively few shed eggs; such adults, however, were large enough to be gravid. These large, nongravid cestodes may represent senescent worms about to be expelled from the gut. Alternatively, low winter temperatures and/or lack of host intestinal contents during winter may alter cestode physiology so adversely that egg

Graph 1. Incidence of parasitism of *Cyprinus carpio* of the Little Sioux River by *Khawia iowensis* during April 1979 through February 1981


100
90
80
70
60
50
40
30
20
10
INCIDENCE (%)
A M J J A S O N J M A M J J A S O N F
1979
MONTH
1980

Graph 2. Mean number of gravid (g), mature, nongravid (m) and immature (i) *Khawia iowensis* per fish (*Cyprinus carpio*) per month (May 1979 through February 1981) in the Little Sioux River

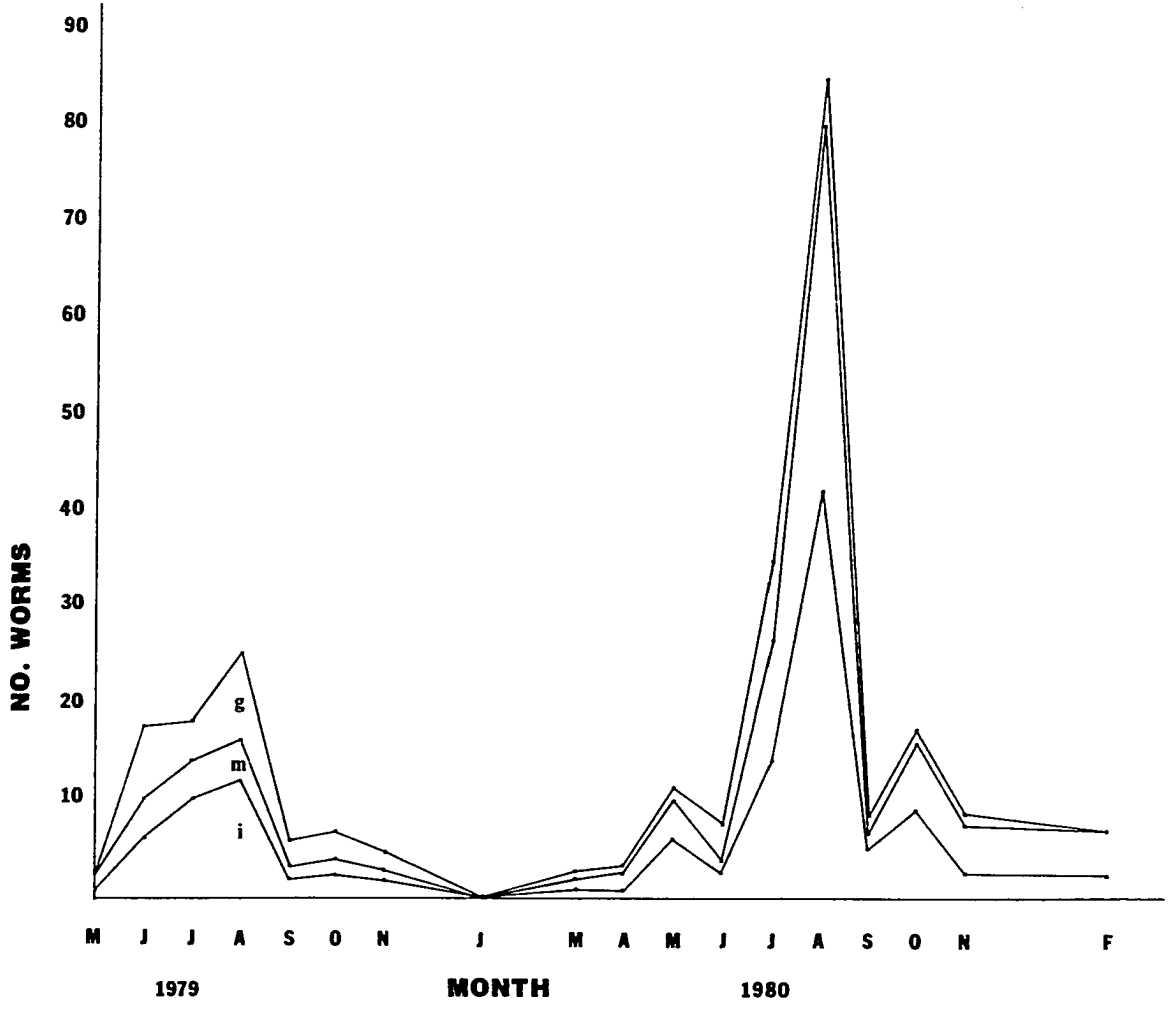
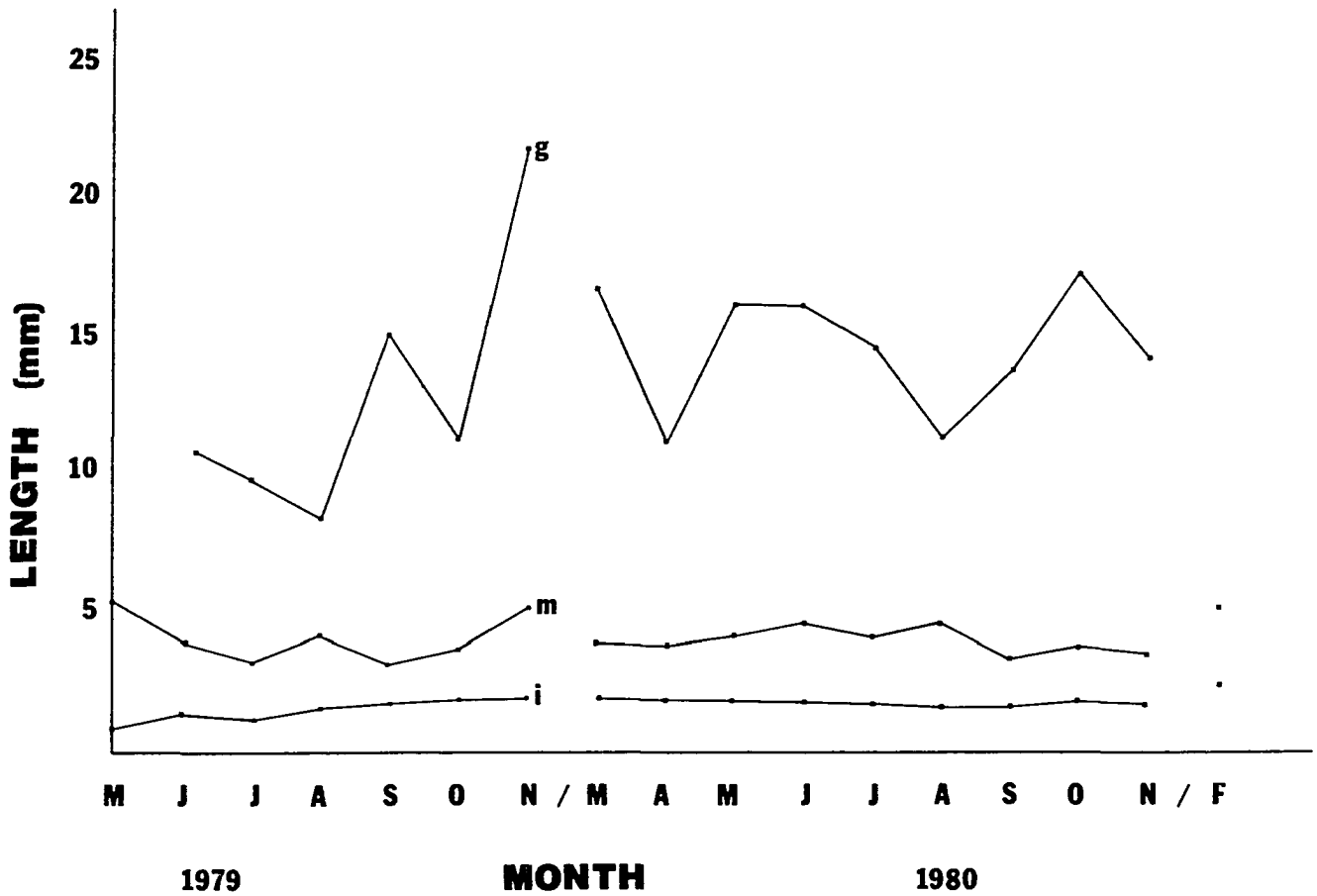

90
80
70
60
50
40
30
20
10
NO. WORMS
g
m
i
M J J A S O N J M A M J J A S O N F
1979
MONTH
1980

Table 4. Seasonal infection patterns of *K. iowensis* in Little Sioux River carp

Season	No. exam.	No. inf. (%)	Mean intensity ± SD	No. of parasites recovered	% gravid worms
Summer (1977)	58	19(33)	4.1 ± 0.8	78	-
Summer (1978)	13	9(69)	13.9 ± 4.1	125	-
Fall	69	62(90)	14.4 ± 2.8	892	-
Spring (1979)	6	3(50)	1.7 ± 0.7	5	0
Summer	54	45(83)	17.9 ± 3.6	805	31
Fall	70	40(57)	5.4 ± 1.0	215	42
Winter (1979-80)	22	0(0)	0	0	0
Spring (1980)	70	48(69)	6.2 ± 1.3	296	6
Summer	76	66(87)	44.7 ± 13.1	2,953	12
Fall	83	63(76)	10.2 ± 1.9	644	7
Winter (1980-81)	23	9(39)	6.9 ± 2.4	62	0

Graph 3. Length (in mm) of gravid (g), mature, nongravid (m) and immature (i) *Khawia iowensis* from *Cyprinus carpio* per month (May 1979 through February 1981) in the Little Sioux River


25
20
15
10
5
LENGTH (mm)
g
m
i
M J J A S O N / M A M J J A S O N / F
1979
MONTH
1980

production by the cestode ceases and is then resumed when temperatures warm in the spring and/or when carp begin feeding again. Williams (1979a) noted the presence of numerous intrauterine ova lacking egg shells in gravid Isoglaridacris wisconsinensis Williams, 1977 collected during winter from fish hosts.

Carp collected from the Little Sioux River during winter and early spring had little or no intestinal contents, indicating that feeding activity is greatly reduced during winter. Huet (1970) confirms that carp cease feeding when water temperatures fall below 5 C.

A number of infected carp were collected in August 1980 and were maintained in the laboratory without food for four days before they were killed and necropsied. Some of the large (11.8-17.6 mm) cestodes recovered at this time resembled senescent worms of winter and early spring in lacking intrauterine eggs. This lends further support to the hypothesis that fish hosts must actively feed in order for cestodes to produce eggs.

Also notable in winter and early spring collections of K. iowensis is the scarcity of immature worms and relatively greater number of mature, nongravid worms from carp. The paucity of immature worms may be related to low acquisition resulting from cessation of feeding during winter months.

Such mature, nongravid worms probably overwintered in carp and had been acquired during the previous fall or summer.

Because procercoids of K. iowensis do not mature in tubificids, Khawia must be ingested by fish as immature worms (Figs. 4 and 5). Infections are acquired in spring as soon as carp begin feeding, and acquisition continues throughout summer and fall months. Reduction in acquisition of immature worms parallels reduction and eventual cessation of feeding as the winter season approaches. During spring and summer months, there is an increase in number of mature and gravid worms that results from growth of previously acquired worms.

In summer, incidence of infection in carp may be as high as 87% with more than 800 cestodes in a single fish. During both field seasons in this study, maximum incidence and mean intensity of infection with K. iowensis occurred in August (Graphs 1 and 2). Although there was some decrease in prevalence of K. iowensis infection with onset of the fall season, the most pronounced change was a reduction of mean intensity. For both field seasons, this significant decrease in mean intensity (Wilcoxon rank sum test, $p < 0.001$) was most obvious between maximum mean intensities of August and lower September mean intensities (Graph 2). Fall incidences and mean intensities gradually declined over the course of

the season.

This reduction in cestode burden between August and September cannot be correlated with cessation of feeding in river carp since C. carpio feed extensively until late fall. However, reduction in worm burden does correspond closely to the time of year when water temperatures in the Little Sioux River normally reach levels that inhibit carp spawning (Scott and Crossman 1973). Perhaps this reduction in worm burden is the result of seasonal hormonal changes in hosts. Another possibility may be some undetected change in diet of carp between August and September. A final and more likely explanation might involve a combination of natural senescence of gravid Khawia and a reduction in acquisition of infective procercoids. Many immature K. iowensis found in carp intestines in late summer and fall may be noninfective worms which, though ingested by carp, did not reach sufficient maturity in oligochaetes to permit their attachment to the carp gut.

Also of interest is the scarcity of gravid worms recorded during the latter part of the 1980-81 field season. No confirmed data are presently available to explain the small numbers of gravid worms recovered, especially in view of the large numbers of mature worms noted during this same period. Of possible importance, however, is the so-called crowding effect seen in many intestinal helminths of mammals

(Kennedy 1975). Because of increased competition for some limited resource, the large cestode suprapopulations in carp of summer and fall 1980 may have resulted in reduced growth and maturation of individual worms.

Apparent differences exist between summer 1979 and summer 1980 mean intensities of K. iowensis of Little Sioux River carp (Graph 2, Table 4). Summer 1979 was preceded by a very severe winter which resulted in considerable winterkill of carp and was presumably detrimental to oligochaete populations; summer 1979 was characterized by considerable rainfall and extensive flooding of the Little Sioux River. During the summer months, carp were collected in sheltered areas normally not inundated and gut contents of such fish were primarily plant materials. Summer 1980, on the other hand, was preceded by a very mild winter with ice on the river for only two months; summer 1980 had little rainfall and the river was reduced to a series of pools. Gut contents of such carp consisted primarily of benthic organisms, including burrowing mayfly larvae, chironomid larvae and tubificids.

Field studies for K. iowensis indicate that a seasonal maturation cycle similar to that of K. sinensis may be occurring. Kulakovskaya (1962a) originally reported that it takes four to six months for K. sinensis to reach sexual

maturity in carp. However, after conducting both field and experimental studies, Sapozhnikov (1972) found that during June and July, K. sinensis reach sexual maturity within 15 days of carp infection. Khawia sinensis increased in size from a procercoid length of less than two mm to as much as ten cm by 20 days post-infection. After about 30 days, K. sinensis were expelled from fish. Khawia acquired by carp in fall were capable of surviving the winter and maturing the next spring. It was thus possible for K. sinensis ingested during fall to remain in carp for six or seven months. During winter months, K. sinensis in carp did not contain intrauterine eggs. It would seem that a similar maturation cycle also occurs with K. icwensis.

Working in carp ponds of the western Ukraine, Kulakovskaya (1962a,c, 1964a,b) has determined that oviposition for K. sinensis occurs primarily in April through May. Development of the procercoid in the tubificid host required two to three months during spring and summer. Prevalence of K. sinensis infection increased from 6.2% in midwinter to 42% in mid-May. It continued to rise in June, reaching 80% prevalence and an intensity of 6.3 parasites per fish. The incidence fell in late August to 28% and intensity of infection also decreased. A few immature K. sinensis were found in carp during winter. Sapozhnikov (1972) and Nakajima and

Egusa (1978) found similar midsummer peaks and midwinter lows for K. sinensis infections in carp ponds of the Moscow and Tokyo regions, respectively. Nakajima and Egusa, however, did not recover any worms from fish between January and May.

Kulakovskaya et al. (1965) found that periodicity of caryophyllideans in carp ponds of the Ukraine is dependent upon a number of parameters (Table 5). According to Kennedy

Table 5. Biotic and abiotic factors influencing seasonal distribution of caryophyllidean cestodes in carp ponds of the Soviet Union [from Kulakovskaya et al. (1965)]

Caryophyllideans
Species present
Species development rates in oligochaete and carp
Tubificids
Species composition
Age distribution of population
Population density
Degree of utilization as food items by carp
Incidence and intensity of infection
Carp
Population density
Age of fish
Incidence and intensity of infection
Pond
Size
Depth
Amount and type of littoral vegetation
Annual drainage and liming
Percent of year fish are present

(1968), the following factors may produce seasonal periodicities within fish hosts: seasonal differences in host feeding habits, seasonal differences in number of infected annelids and seasonal changes in host resistance. Seasonal periodicity of caryophyllideans is obviously a very complex phenomenon.

Kennedy and Walker (1969) and Kennedy (1971) have presented evidence for a seasonal immune response by Leuciscus to infections by C. laticeps. Such a mechanism explains seasonal distributions for caryophyllideans (such as C. laticeps and G. catostomi) which exhibit midwinter seasonal population peaks at the time when the fish immune responses are depressed and which exhibit midsummer infrapopulation lows when the immune response is most effective. Such an hypothesis, is not, however, appropriate for explaining the seasonal distribution of K. iowensis.

In the Little Sioux River, both P. bulbocolli and Capillaria sp. lack a seasonal infection distribution in carp (Graph 4, Tables 6 and 7). Immature and gravid worms of both species were recovered during all seasons of both collecting years. That Pomphorhynchus does not display seasonal peridocity is confirmed by the literature, including studies by Muzzall (1980b), who worked in New Hampshire with P. bulbocolli from white suckers; Hine and Kennedy (1974), working with P. laevis (Müller, 1776) from L. leuciscus in England; and Rumpus (1975), also working with

Graph 4. Incidence of parasitism of Cyprinus carpio by Pomphorhynchus bulbocolli (P) and Capillaria sp. (C) during May 1979 through February 1981 in the Little Sioux River

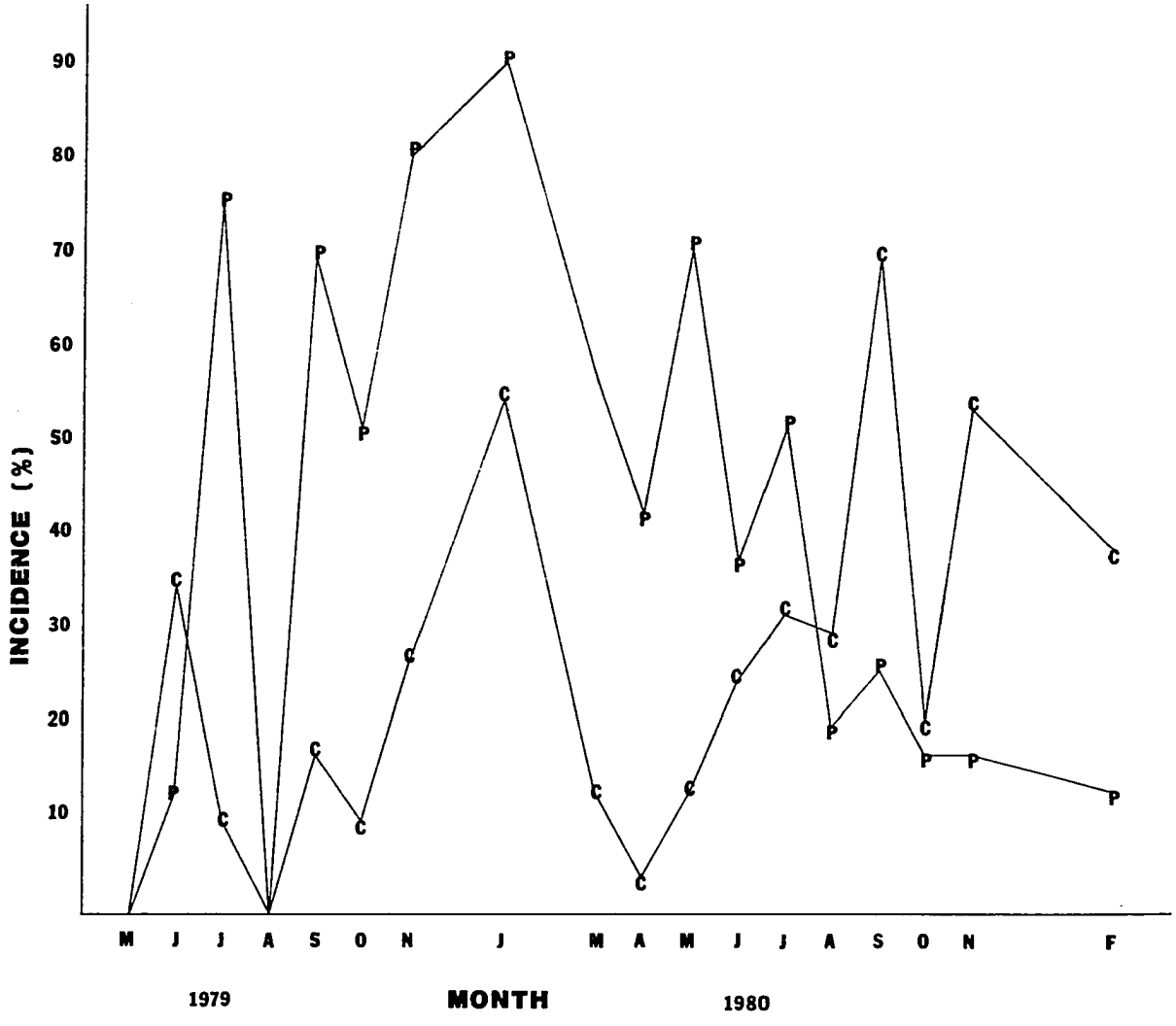

INCIDENCE (%)
90
80
70
60
50
40
30
20
10
M J J A S O N J M A M J J A S O N F
1979
MONTH
1980

Table 6. Seasonal infection patterns of *P. bulbocolli* in Little Sioux River carp

Season	Number examined	Number infected (%)	Mean intensity ±SD	Number of parasites recovered	Range
Summer (1977)	58	0	0	0	
Summer (1978)	13	1(8)	4.0	4	
Fall	69	7(10)	3.9 ± 1.9	27	1-15
Spring (1979)	6	0	0	0	
Summer	54	25(46)	6.8 ± 1.8	170	1-30
Fall	70	48(83)	4.1 ± 0.7	199	1-24
Winter (1979-80)	22	20(91)	5.3 ± 0.7	106	1-14
Spring (1980)	70	40(57)	5.5 ± 1.7	220	1-64
Summer	76	27(36)	5.2 ± 2.7	140	1-61
Fall	83	15(18)	4.3 ± 1.4	65	1-22
Winter (1980-81)	23	3(13)	4.7 ± 3.7	14	1-12

Table 7. Seasonal infection patterns of *Capillaria* sp. in Little Sioux River carp

Season	Number examined	Number infected (%)	Mean intensity ±SD	Number of parasites recovered	Range
Summer (1977)	58	0	0	0	
Summer (1978)	13	1(8)	3.0	3	
Fall	69	0	0	0	
Spring (1979)	6	0	0	0	
Summer	54	11(20)	7.6 ± 2.5	84	1-30
Fall	70	13(19)	5.1 ± 1.7	66	1-21
Winter (1979-81)	22	12(54)	3.3 ± 1.1	40	1-11
Spring (1980)	70	7(10)	12.0 ± 6.2	84	1-42
Summer	76	22(29)	5.3 ± 2.0	117	1-41
Fall	83	43(52)	9.3 ± 1.8	399	1-65
Winter (1980-81)	23	9(39)	8.8 ± 3.2	79	1-33

P. laevis from Cottus gobio (L.) in England.

Intestinal Distribution

Although specimens of K. iowensis are found throughout the entire length of the carp intestine (Graph 5a, Table 8), most are recovered from the first third. In carp from both habitats, intestinal segments one, two and three were more heavily parasitized by K. iowensis than segments four through eight (chi-square test, $p < 0.001$). Mean intensities in segments one through three were not significantly different. Attachment distributions for K. iowensis are essentially the same in both heavy ($x > 50$ worms) and light ($x < 50$ worms) infections (Graph 5b).

Immature and mature, nongravid Khawia are found almost exclusively in the anterior quarter of the gut. The location of gravid worms is more variable, many being recovered from the posterior half of the intestine. Worms recovered from the posterior half of the gut, especially smaller specimens, are often not attached to the gut mucosa and are usually wholly incorporated into the fecal string. Those in the anterior half of the gut are normally intimately attached to the gut (Fig. 6). The strobila of a gravid worm is frequently folded back upon itself two or three times (Fig. 7). Smaller worms usually have their stobila oriented longitudinally within the gut lumen, with the hind end of the worm

Graph 5. a. Distribution of attachment sites of *Khawia iowensis*, *Pomphorhynchus bulbocolli* and *Capillaria* sp. within the intestinal tract of *Cyprinus carpio*. b. Distribution of attachment sites of *K. iowensis* within the intestinal tract of heavily ($x > 50$) and lightly ($x < 50$) infected *C. carpio*. c. Distribution of attachment sites of *Capillaria* sp. within the intestinal tract of heavily ($x > 100$) and lightly ($x < 100$) infected *C. carpio*. (Graphs based on combined data from Little Sioux River and Lower Gar Lake carp)

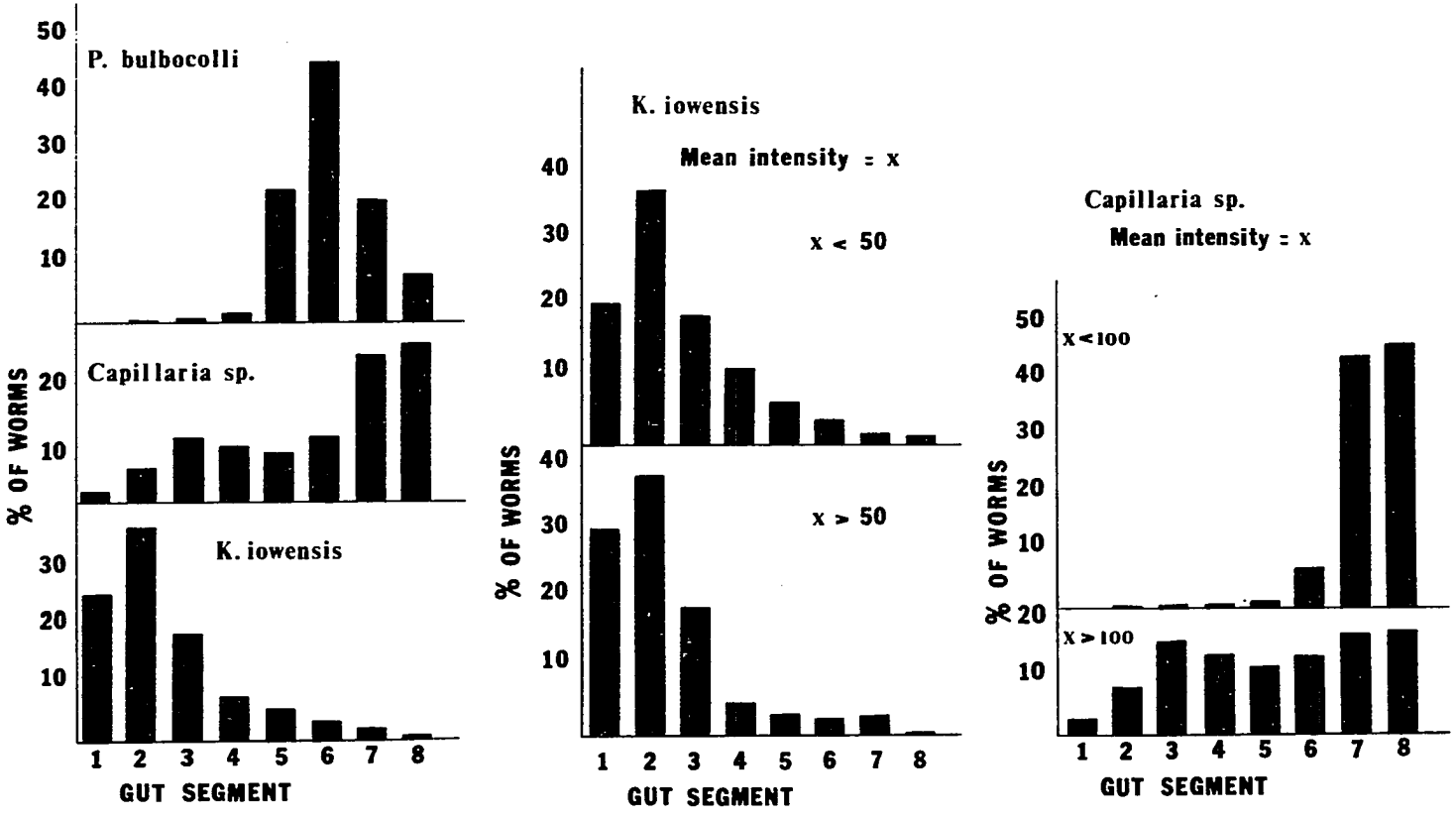

P. bulbocolli
Capillaria sp.
K. iowensis
% OF WORMS
GUT SEGMENT
K. iowensis
Mean intensity = x
x < 50
x > 50
% OF WORMS
GUT SEGMENT
Capillaria sp.
Mean intensity = x
x<100
x>100
% OF WORMS
GUT SEGMENT

Table 8. Total frequency of occurrence and mean intensity ±SE of each helminth species from Little Sioux River and Lower Gar Lake carp, by intestinal segment

Locality	Intestinal segment							
	1	2	3	4	5	6	7	8
Little Sioux River								
K. iowensis	1,230 10.1±2.1	1,792 9.41±2.1	954 6.1±1.5	430 3.6±0.4	272 3.7±0.6	147 3.1±0.8	115 3.4±1.6	36 1.8±0.3
A. huronensis	18 6.0±2.6	10 10	0	0	0	0	0	0
P. bulbocolli	0	1 1	1 1	14 1.8±0.4	192 2.8±0.5	396 3.6±0.5	164 2.4±0.4	41 1.6±0.3
Capillaria sp.	0	1 1	1 1	7 2.3±0.7	7 1.0±0.0	82 3.0±0.9	407 5.3±0.9	343 4.6±0.5
Lower Gar Lake								
K. iowensis	70 2.5±0.5	138 4.1±1.1	66 2.4±0.6	27 1.4±0.2	8 1.6±0.4	10 1.4±0.3	4 1.3±0.3	2 1.0±0.0
A. huronensis	13 1.6±0.4	4 1.3±0.3	0	0	0	0	0	0
P. bulbocolli	0	1 1	5 1.7±0.7	12 2.4±1.2	327 11.7±6.9	553 8.5±3.1	278 4.3±0.8	124 3.4±1.0
Capillaria sp.	132 16.5±1.1	515 46.8±14.7	1,002 83.5±15.4	850 70.8±13.9	745 57.3±19.7	860 43.0±13.8	1,320 40.0±12.6	1,454 34.6±10.1

posteriad to the scolex. Unlike some other caryophyllideans (H. nodulosa and Monobothrium ulmeri Calentine and Mackiewicz, 1966), K. iowensis does not occur in conspicuous clusters, but individuals are usually distributed uniformly throughout a given segment of gut.

Worms of all sizes, when dislodged from the anterior portion of the gut and placed in lake water, usually exhibit considerable movement. This involves alternate contraction and elongation of the body plus contraction and expansion of individual fimbriae of the scolex. Many worms removed from the posterior part of the gut were much more sluggish in their movements and some exhibited no movement at all, suggesting they were dead. Two large specimens from the posterior gut exhibited active scolex movement, but the posterior three quarters of each worm was completely opaque and exhibited no movement. Subsequent staining and mounting of these two Khawia indicated that the bodies had decomposed and had numerous tegumental ruptures. The scoleces and necks of both worms, however, appeared completely normal. It would thus seem that the posterior portion of the carp intestine is not the optimal habitat for K. iowensis. Most worms found in the hind portion of the gut may represent moribund and dead worms in the process of being shed from the gut. That gravid Khawia are often found more posteriad than immature cestodes provides some

evidence for an ontogenetic shift in attachment sites [see Holmes (1973)].

Capillaria sp. are distributed throughout the length of the gut (Graph 5a, Table 8); however, when the data are graphed according to heavy ($x > 100$ worms) and light ($x < 100$ worms) (Graph 5c) infections, it is evident that Capillaria exhibited a significant preference for segments seven and eight in light infections (chi-square test, $p < 0.001$) as opposed to segments one through six. Presumably because of intraspecific competition for space and/or nutrients, there is a forward shift in the attachment distribution of the nematodes in relatively heavy infections.

Similarly, P. bulbocolli exhibited a significant preference for intestinal segments five, six and seven (chi-square test, $p < 0.001$) as opposed to segments one, two, three, four and eight. Mean intensities in segments five, six and seven were not significantly different. It was not unusual in heavy infections of this relatively large acanthocephalan to find as many as 200 or more worms in a three or four cm stretch of gut. For P. bulbocolli, no evidence for intraspecific competition, i.e., shift in attachment distribution, similar to that seen for Capillaria, was found. There was no evidence of a posterior or anterior seasonal movement by P. bulbocolli or Capillaria sp.

In working with white sucker intestinal helminths,

Muzzall (1980b) divided the gut into five equal segments and found that segment four was more heavily parasitized by P. bulbocolli than segments three and five. Muzzall did find that P. bulbocolli normally occurred in segment four when hosts were lightly infected, but when mean intensity was high, worms were also found in segments two through three and five.

Even though Pomphorhynchus and Capillaria overlap in their longitudinal distribution, an argument can be made for radial segregation of the infrapopulations within the gut with regard to nutrient procurement (Fig. 8). In P. bulbocolli, the trunk, which is the main nutrient absorbing part of the body, lies free in the lumen of the gut; the mouth of Capillaria is deeply buried within the submucosa.

To date, no interspecific interactions of consequence have been demonstrated between the three predominant intestinal helminths of carp. Neither competitive exclusion or interactive site segregation [see Holmes (1973, 1979)] have been demonstrated. Since each species appears to occupy restricted, distinct microhabitats which do not change in the presence or absence of the other species, selective site segregation appears to be occurring.

Effect of Host Size

Correlation analyses indicated that both prevalence ($r = 0.83$) and mean intensity ($r = 0.23$) of *K. iowensis* increased as fish length increased in the Little Sioux River (Table 9). Such increases are probably the result of increases in numbers of infected oligochaetes eaten by larger fish. Also, as fish increase in length, so does the intestine; therefore, there is more space and a larger surface area to attach to and occupy. Young-of-the-year carp (less than ten cm in length) are primarily planktivorous (Huet 1970) and their relatively light cestode burdens would be expected. Upon switching to a benthic feeding regimen, the cestode infrapopulations increased dramatically.

Little Sioux River carp, though smaller, were less heavily infected than larger fish; these small carp harbored a proportionately greater percentage of gravid worms (Table 9). Of particular interest is the presence of numerous gravid *K. iowensis* from immature carp during summer and fall 1980 and conspicuous lack of gravid cestodes from mature carp during the same time period. The possible role of host hormonal levels has already been mentioned (see Seasonal Infection Patterns).

Table 9. Prevalence and mean intensity of K. iowensis recovered from 520 Little Sioux River carp and 253 Lower Gar Lake carp of various length classes (cm), examined in the total sampling period

Fish length class	Mean fish length	Number examined	Number infected (%)	Mean intensity ±SD	Number parasites recovered	% Gravid worms[a]
Little Sioux River						
1-10	8.7	48	12(25)	3.4±0.9	41	-
11-20	15.2	45	28(62)	6.5±2.8	181	25
21-30	27.8	127	95(75)	12.2±1.7	1,160	40
31-40	34.5	170	130(76)	14.7±2.3	1,916	11
41-50	44.6	82	59(72)	17.4±4.7	1,029	6
51-60	55.5	26	23(88)	67.3±34.8	1,548	11
61-70	64.5	20	14(70)	12.7±4.1	178	22
71-80	71	1	1(100)	2	2	50
81-90	83	1	1(100)	16	16	63
Lower Gar Lake						
11-20	17	1	0	0	0	
21-30	27.5	14	10(71)	4.3±1.3	43	
31-40	36.0	112	51(46)	5.2±0.7	263	
41-50	45.2	44	21(48)	5.4±1.2	113	
51-60	55.6	39	26(67)	6.6±1.3	171	
61-70	63.6	26	10(38)	13.7±7.2	137	
71-80	75.0	12	3(25)	2.7±1.2	8	
81-90	82.0	4	2(50)	1.5±0.5	3	
91-100	93	1	0	0	0	

[a]Based on 1979 through 1981 collections from Little Sioux River.

Lawrence (1970) reported that white suckers in the five and five plus year groups had the heaviest infections of G. catostomi. Muzzall (1980a) also reported that prevalence and mean intensity of G. catostomi increased as host length increased. Kanaev (1956) found that the number of C. fimbriceps decreased as carp became older and suggested this to be related to an age related immunity. Rakova (1953) reported that prevalence of C. laticeps decreased with age of chub, Leuciscus idus (L.) and attributed this decrease to nonbenthic habits of older fish.

For Capillaria sp., there appears to be little relationship between infection rates and carp size (Table 11). However, correlation analyses indicated that prevalence ($r = 0.78$) and mean intensity ($r = 0.60$) of P. bulbocolli increased as fish length increased in the Little Sioux River (Table 10). The acanthocephalan and nematode were rarely encountered in fish less than ten cm in length. Carp of larger size classes harbored variable numbers of each helminth species. Lawrence (1970) found no relationship between P. bulbocolli infection levels and white sucker age. However, Hine and Kennedy (1974) and Rumpus (1975), both working with P. laevis and Muzzall (1980b), working with P. bulbocolli, found that the mean acanthocephalan burden increased with host age and size.

Table 10. Prevalence and mean intensity of P. bulbocolli recovered from 520 Little Sioux River carp and 253 Lower Gar Lake carp of various length classes (cm), examined in the total sampling period

Fish length class	Mean fish length	Number examined	Number infected (%)	Mean intensity ±SD	Number parasites recovered
Little Sioux River					
1-10	8.7	48	0	0	0
11-20	15.2	45	13(29)	2.6±0.7	34
21-30	27.8	127	35(28)	2.9±0.4	103
31-40	34.5	170	58(34)	6.7±1.4	387
41-50	44.6	82	38(46)	5.4±1.7	204
51-60	55.5	26	13(50)	2.7±0.5	35
61-70	64.5	20	8(40)	9.4±3.3	75
71-80	71	1	0	0	0
81-90	83	1	0	0	0
Lower Gar Lake					
11-20	17	1	0	0	0
21-30	27.5	14	10(71)	8.0±3.8	80
31-40	36.0	112	74(66)	10.4±2.5	768
41-50	45.2	44	33(75)	20.2±12.1	666
51-60	55.6	39	29(74)	9.2±3.6	267
61-70	63.6	26	17(65)	10.9±3.3	186
71-80	75.0	12	7(58)	17.4±11.0	122
81-90	82.0	4	1(25)	3	3
91-100	93	1	1(100)	19	19

Table 11. Prevalence and mean intensity of Capillaria sp. recovered from 520 Little Sioux River carp and 253 Lower Gar Lake carp of various length classes (cm), examined in the total sampling period

Fish length class	Mean fish length	Number examined	Number infected (%)	Mean intensity ±SD	Number parasites recovered
Little Sioux River					
1-10	8.7	48	2(4)	5.0±0.0	10
11-20	15.2	45	25(56)	8.0±1.4	199
21-30	27.8	127	22(17)	6.1±2.1	134
31-40	34.5	170	29(17)	11.4±2.8	332
41-50	44.6	82	16(20)	4.8±1.0	76
51-60	55.5	26	8(31)	2.9±1.2	23
61-70	64.5	20	4(20)	14.5±9.0	58
71-80	71	1	0	0	0
81-90	83	1	0	0	0
Lower Gar Lake					
11-20	17	1	0	0	0
21-30	27.5	14	3(21)	25.7±22.7	77
31-40	36.0	112	27(24)	112.3±53.3	3,033
41-50	45.2	44	10(23)	82.4±44.3	824
51-60	55.6	39	4(10)	20.5±9.9	82
61-70	63.6	26	6(23)	24.5±11.1	147
71-80	75.0	12	1(8)	7	7
81-90	82.0	4	0	0	0
91-100	93	1	1(100)	1	1

Effect of Host Sex

Host sex was not found to be a significant factor in determining the infection rates of K. iowensis (Table 12), P. bulbocolli or Capillaria sp. Similar results were reported by Lawrence (1970) and Muzzall (1980a,b).

Grimes and Miller (1976) reported a relationship between host sex and mean intensity of M. ulmeri. Male creek chubsuckers, Erimyzon oblongus (Mitchill) had higher intensities of M. ulmeri than did females during winter and early spring. This corresponded with testicular development and formation of breeding tubercles in males. Worm burdens decreased with degeneration of tubercles. Female chubsuckers acquired progressively higher intensities during early spring and by June surpassed those of males. This increase corresponded closely to the development of the ovaries.

In a survey of roach, Rutilus rutilus (L.), Borgström and Halvorsen (1968) found Caryophyllaeides fennica (Schneider, 1902) only in female fish. They attributed the lack of C. fennica in male roach to physiological differences in the sexes.

Table 12. Prevalence and mean intensity of Khawia iowensis infections in male and female Cyprinus carpio from Little Sioux River and Lower Gar Lake collections during 1978-1980

Collecting site Year Sex	Prevalence	Mean intensity
Little Sioux River		
1980		
Males	75.2%	19.6 worms
Females	75.6	29.0[a]
1979		
Males	71.4	12.1
Females	71.4	11.5
1978		
Males	87.9	18.3
Females	89.3	13.3
Lower Gar Lake		
1979		
Males	44.6	5.3
Females	46.7	6.0
1978		
Males	59.4	7.4
Females	44.4	5.3

[a]Without the one female carp harboring 815 K. iowensis, the mean intensity would be 17.3.

Experimental Studies

Following the discovery in July 1978 of K. iowensis in A. pigueti, experimentally infected oligochaetes harboring large procercoids of this species which were at least 70 days old were fed to 30 small specimens of C. carpio (between 20 and 25 cm in total length) and 15 small specimens of goldfish, C. auratus (between ten and 14 cm in length). Each of these fish was fed one to 15 infected tubificids. Although fish were examined as soon as 48 hr post-feeding, no cestodes, live or dead, were recovered. Feeding experiments were conducted at a time of year when natural infection occurred in carp.

Despite these unsuccessful feeding experiments, it nonetheless appears that carp acquire infections as a result of their feeding activities. Examination of intestinal contents of carp collected during March through November shows that these fish ingest tubificids during these months. The possibility of a second intermediate host for Khawia seems slight, indeed. A number of investigators have found that tubificids are able to transmit infections of caryophyllideans directly to fish hosts (Kennedy 1971, Mudry and Arai 1973a). Kulakovskaya (1962a) appears to have succeeded in infecting carp with K. sinensis; regrettably, there are no detailed data of her experiments.

Sapozhnikov (1972) has recently succeeded in experimentally infecting carp with K. sinensis. In Sapozhnikov's study, carp were maintained in ponds within specially constructed cages and fed "zooplankton and mixed feed." One-hundred percent of the experimental carp became infected after being fed oligochaetes harboring "infective procercoids." Within 15 days of exposure, gravid K. sinensis between 5.6 and 7.8 cm were recovered. The length of gravid worms recovered increased through day 25. After 30 days, dissection of experimental carp revealed no Khawia. No control fish were infected at any time.

Of probable significance in fish feeding experiments is the use of "infective" procercoids. In the Russian literature, repeated reference is made to "infective" (or "infectious") and "uninfective" (or "young") procercoids (Kulakovskaya 1962a, Kupchinskaya 1972, Sapozhnikov 1972, 1976). These two types are not distinguished in the literature but may be based upon morphological and/or age criteria. Perhaps in the feeding experiments with K. iowensis, "uninfective" procercoids were used. Further studies on the infection of carp and goldfish by K. iowensis are in progress.

GRAVID ADULTS

The morphology of gravid K. iowensis was presented by Calentine and Ulmer (1961). Because of several recent publications redescribing a number of the previously less well known species of Khawia, it was thought that a re-examination of the taxa of the genus parasitizing carp might be appropriate. For this reason, wholemount preparations of ten gravid K. iowensis (eight from the Little Sioux River and two from the Iowa River) were critically analyzed (Fig. 9). A number of sectioned worms were also examined to elucidate structures not clear in wholemount preparations. The specific diagnosis is given below.

> Khawia iowensis. With characters of genus. Gravid adults 17 mm (9.8-26.2) by 1.5 mm (0.7-2.0). Scolex 1.32 mm (0.8-1.9) wide and frilled with approximately 9 (7-12) fimbriae. Neck distinct. Body oval, flattened dorso-ventrally. Parenchymal muscles poorly developed.
>
> Testes numerous, averaging 318 (226-367), extending from 1.21 mm (0.5-2.0) behind anterior margin of scolex to level of cirrus pouch. Testes spherical or elliptical, averaging 0.16 by 0.14 mm in anterior region and 0.20 by 0.18 mm in posterior region. Vas deferens convoluted anterior to cirrus pouch. Cirrus pouch large, measuring 0.54 by 0.41 mm. Seminal vesicle lacking. Common genital atrium present, located approximately 3.9 mm (2.2-5.5) from posterior of worm.
>
> Vitellaria spherical or elliptical, averaging 0.07 by 0.06 mm in anterior region and 0.12 by 0.09 mm in posterior region, beginning 1.17 mm (0.6-1.8) from anterior margin of scolex. In region from neck to cirrus pouch, vitellaria cortical, encircling entire body. In region of uterine folds and ovarian wings, vitellaria cortical, but limited to lateral bands of varying follicle numbers. Postovarian vitellaria present,

in both cortical and medullary parenchyma.

Ovary H-shaped, approximately 1.7 mm (1.0-2.0) in length and 1.0 mm (0.7-1.3) in width, entirely medullary. Uterus not extending anterior to cirrus pouch. Uterus uniting with vagina to form utero-vaginal canal. Uterine glands present, well developed, beginning just as the uterus begins to course anteriorly and extending to within several uterine loops of utero-vaginal junction. Vagina expanding into large seminal receptacle, 0.5 by 0.2 mm, which passes dorsal to ovarian isthmus. Intrauterine eggs measure 57 by 46 μm, with or without spinose shells.

Parasitic in anterior half of intestine of Cyprinus carpio in North America.

Scolex and neck morphology vary greatly, especially between material fixed in cold formalin and hot formalin fixed material (Fig. 10). Considerably more fimbriae are evident in cold formalin fixed worms. The neck is also indistinct for K. iowensis killed in cold formalin. In examining over 6,000 K. iowensis in the present study, no examples similar to those described by Calentine and Ulmer (1961) of the frilled scolex being inverted into a pit were observed. In fixed specimens, the terminal excretory bladder is small (59 by 77 μm) and opens into an indentation at the posterior of the body (Fig. 11). Between eight and 14 excretory ducts empty into the bladder. The excretory system consists of an anastomosing system of canals consisting of 12 to 15 major longitudinal channels (Fig. 12).

The tegument is approximately five μm thick. Longitudinal fibers are more prominent than circular fibers in the poorly developed parenchymal musculature. Dorso-ventral

muscle fibers are present in the medullary region. Four major longitudinal muscle bundles are prominent in the neck region. Also evident in the medullary parenchyma of the neck and extending posteriorly into the anterior vitelline and testicular fields, is the well-developed "Fraserzellenstrange" [see Mackiewicz (1972)]. No ducts of any type are associated with the Fraserzellenstrange.

Testes, usually somewhat obscured by vitellaria and appearing in two longitudinal rows, begin near the anterior end. These rows are more distinct in younger worms. Posteriorly, such rows become less distinct. In single cross sections, three to seven testes are usually present. The convoluted vas deferens measures approximately 40 μm where it enters the muscular cirrus pouch (sac). Neither an internal or external seminal vesicle is present. The male genital pore opens into a small, ventral, common genital atrium. The utero-vaginal aperture also opens into this atrium immediately posterior to the male opening. The structure of the female reproductive system is identical to that described by Calentine and Ulmer (1961).

Vitellaria begin in the neck region, appearing as two lateral bands that rapidly become more extensive and form layers completely encircling the testes. This surrounding layer continues to the level of the cirrus pouch. In the

region of the uterine coils, vitellaria appear as two lateral bands of follicles. More posteriorly, a varying number of follicles is found lateral to each ovarian wing. A number of postovarian vitellaria overlap the posterior margins of the posterior ovarian wings.

Gravid specimens of K. iowensis closely resemble those of K. japonensis. Comparison of measurements for Yamaguti's (1934) K. japonensis, Calentine and Ulmer's (1961) K. iowensis and Khawia of the present study reveals remarkable similarity between the two species (Table 13). Calentine and Ulmer separated the two species primarily on the basis of distribution of testes. In K. japonensis, the testes, in cross section, appear in distinct dorsal and ventral layers, seven testes per layer; in K. iowensis, the testes were reported as appearing in a single layer of two to six per layer, as seen in cross section.

In the present study, examination of sectioned K. iowensis has revealed, especially in the posterior regions of testes distribution, that there are usually areas in which testes overlap one another longitudinally, giving the impression of two layers. In fact, numerous groups of testes are seen in which one testis lies entirely above the other, forming two distinct layers.

More recent descriptions of K. japonensis by Bykhovskaya-Pavlovskaya et al. (1962) and Demshin (1978) reveal that

Table 13. Comparison of gravid *Khawia iowensis*, *K. japonensis* and *K. sinensis* from *Cyprinus carpio* (measurements in mm, except where indicated)

Species	*K. iowensis*	*K. iowensis*	*K. iowensis*	*K. japonensis*	*K. japonensis*	*K. japonensis*
Length	11-26	14-48	9.9-26.2	10-20+	23-40	25-45
Width	1.0-2.1	1.0-3.2	0.7-3.2	0.87	1.5-2.7	2-3
Number of testes	328-490	235-513	226-367	not given	not given	not given
Cirrus sac	0.66 X 0.61 circular	0.60-0.69 X 0.60-0.69 circular	0.54 X 0.41 circular-elliptical	0.50 X 0.44 elliptical	0.5 elliptical	0.95 X 1.00 circular
Seminal vesicle	no	not given	no	"small internal seminal vesicle"	"small internal seminal vesicle"	not given
Vitellaria	0.19 X 0.13	not given	0.11 X 0.08	not given	not given	0.13-0.22 X 0.13-0.28
Posterior extent of preovarian vitellaria	adjacent to anterior ovarian wings	adjacent to ovary, often extend laterally to post-ovarian vitellaria	adjacent to ovary, often extend laterally to post-ovarian vitellaria	extending laterally to post-overian vitellaria	extending laterally to post-overian vitellaria	extending laterally to post-ovarian vitellaria

Table 13 (Continued)

Species	K. iowensis	K. iowensis	K. iowensis	K. japonensis	K. japonensis	K. japonensis
Extent of postovarian vitellaria	does not obscure posterior ovarian wings	does not obscure posterior ovarian wings	overlaps posterior ends of posterior ovarian wings	overlaps posterior ends of posterior ovarian wings	overlaps posterior ends of posterior ovarian wings	does not obscure posterior ovarian wings
Eggs (μm)	54 X 42	not given	57 X 46	48-57 X 36-42	51-58 X 38-42	56-62 X 42-50
Reference and location	Calentine & Ulmer (1961) Iowa, U.S.A.	Williams & Sutherland (1981) Iowa, Wisconsin, U.S.A.	This paper Iowa, U.S.A.	Yamaguti (1934) Lake Biwa Japan	Bykhovskaya-Pavlovskaya et al. (1962) Amur River U.S.S.R.	Demshin (1978) Amur River U.S.S.R.

Species	K. sinensis	K. sinensis	K. sinensis	K. sinensis	K. sinensis	K. sinensis
Length	55-95	48-83	80-170	50-80	11.0-16.2	62-69
Width	1.1-5.0	2.5-3.0	3.5-5.0	2.0	0.8-2.4	1.1-1.2
Number of testes	not given	not given	not given	not given	not given	467-494
Cirrus sac	0.56 X 0.81 elliptical	0.76 X 0.80 elliptical	0.76 X 0.80 circular	0.80-0.96 X 0.64-0.72 elliptical	0.52-0.68 X 0.40-0.56 elliptical	0.46-0.51 X 0.65-0.68 elliptical

Table 13 (Continued)

Species	K. sinensis	K. sinensis	K. sinensis	K. sinensis	K. sinensis	K. sinensis
Seminal vesicle	not given	not given	not given	not given	not given	not given
Vitellaria	not given	not given	not given	not given	not given	0.08-0.96 X 0.10-1.14
Posterior extent of preovarian vitellaria	adjacent to cirrus sac	adjacent to cirrus sac	adjacent to cirrus sac	adjacent to cirrus sac	not given	adjacent to cirrus sac
Extent of postovarian vitellaria	partially obscures posterior ovarian wings	partially obscures posterior ovarian wings	partially obscures posterior ovarian wings	partially obscures posterior ovarian wings	not given	partially obscures posterior ovarian wings
Eggs (µm)	46-48 X 26-30	42-48 X 25-30	42-48 X 25-30	48-51 X 29-33	44-50 X 22-34	not given
Reference and location	Hsü (1935) Peking China	Bykhovskaya-Pavlovskaya et al. (1962) widespread U.S.S.R.	Bauer et al. (1969) widespread U.S.S.R.	Murai & Molnar (1975) Hungary	Nakajima & Egusa (1978) Tokyo Japan	Williams & Sutherland (1981) Iowa U.S.A.

gravid worms of this species may reach 45 mm in length. In the present study, no K. iowensis longer than 34.8 mm were recovered. However, Williams and Sutherland (1981) report specimens of K. iowensis reaching 48 mm in length.

Other differences between K. iowensis and K. japonensis include presence or absence of an internal seminal vesicle and the distribution of vitellaria along either side of the ovary. By definition, an internal seminal vesicle is a swollen area of the vas deferens, functioning in sperm storage and located entirely within the cirrus pouch. Yamaguti (1934) and Bykhovskaya-Pavlovskaya et al. (1962) possibly quoting Yamaguti, state that K. japonensis has a "small" internal seminal vesicle. Specimens of Khawia from Iowa carp have the vas deferens highly convoluted within the cirrus pouch but there is little discernible increase in diameter of the duct. Sperm storage probably occurs in this convoluted stretch of vas deferens. The internal seminal vesicle of K. japonensis is also highly convoluted within the cirrus pouch. The presence or absence of an internal seminal vesicle in these two species appears to be merely a semantic argument. Also, distribution of vitellaria surrounding the ovary has also been shown to be highly variable in K. iowensis (Williams and Sutherland 1981 and this dissertation).

It would seem at the present time that there are few morphological characters, if any, by which K. iowensis and K. japonensis can be separated. Recent studies on the life cycles of K. japonensis by Demshin (1978) and K. iowensis (this dissertation) provide few additional criteria by which to separate the two species. Demshin found K. japonensis to develop in L. udekemianus; no reference is made to attempts at infecting other oligochaete species. Procercoids of K. iowensis were found to develop only in A. pigueti in the present study. Both L. udekemianus and A. pigueti are cosmopolitan in their distribution (Brinkhust and Jamieson 1971). Since L. udekemianus were not collected during the present study in sufficient numbers to establish cultures for feeding experiments, their role as intermediate hosts of K. iowensis remains unknown. Steps to establish cultures of L. udekemianus are presently being undertaken.

Aulodrilus pigueti is a predominant oligochaete in Iowa, where L. udekemianus is only infrequently recovered. Calentine (Biology Department, University of Wisconsin, River Falls, personal communication) has only infrequently recovered A. pigueti from Wisconsin streams, whereas L. udekemianus occurs quite commonly. If K. iowensis and K. japonensis are, in fact, one and the same species, it may be that A. pigueti serves as the main intermediate host in the turbid slow-moving streams of Iowa and

L. udekemianus is the required oligochaete host in the predominantly faster moving waters of Wisconsin.

Until the status of L. udekemianus as an intermediate host of K. iowensis can be determined, it is suggested that K. iowensis and K. japonensis be retained as separate species. Should K. iowensis utilize L. udekemianus as an oligochaete host, there appears to be only the difference in geographical distribution by which to separate the two species. With no present interactions between the gene pools of the North American and Asian Khawia, it is likely that speciation on the part of the two populations may now have occurred.

Determination of the specific diagnosis of Capillaria sp. (Figs. 13-19) is in progress. Hoffman (Fish Farming Experimental Station, Stuttgart, Arkansas, personal communication) has recorded C. catostomi Pearse, 1924 in a number of cyprinids from Arkansas and has suggested that the Capillaria from carp of the present study may also be C. catostomi. Pomphorhynchus bulbocolli has been described morphologicaly in a number of papers, but to my knowledge no figures of the female exist. The male and female of P. bulbocolli are represented in Figs. 20-21.

PATHOLOGY IN OLIGOCHAETES AND FISH

Oligochaete Pathology Elicited by Caryophyllideans

The effect of caryophyllidean larvae on their annelid host is highly variable (Mackiewicz 1972). Kennedy (1965) found that A. limnodrili (Yamaguti, 1934) caused rupture of the annelid body when the cestode became gravid. Calentine (1967) noted that G. catostomi also ruptured the body wall and induced death of infected Tubifex. Kulakovskaya (1962a) found that heavy infections (more than ten procercoids) resulted in high host mortality. Infection with H. nodulosa and G. catostomi, according to McCrae (1960), resulted in loss of the posterior segments of L. hoffmeisteri. Calentine et al. (1970) indicated that uninfected oligochaetes lived longer than infected ones.

Calentine (1963) observed that posterior seminal vesicles of L. hoffmeisteri infected with A. iowensis procercoids rarely contained spermatozoa. The anterior seminal vesicles, however, harbored few procercoids and usually contained spermatozoa. Even though ovaries of parasitized tubificids appeared normal, ova were rarely produced. Hyperplasia of posterior seminal vesicles occurred in infected oligochaetes and these organs increased in size and

extended posteriad with growth of the procercoid.

Other examples of caryophyllideans causing sterilization of the annelid host were reported by Kulakovskaya (1962a) for K. sinensis and by Calentine (1967) for G. catostomi. Infection with C. laticeps, on the other hand, merely depressed growth rate and delayed the breeding period of P. barbatus according to Kennedy (1969).

Procercoid development may damage structures other than reproductive organs. Larvae of G. catostomi and Monobothrium hunteri Mackiewicz, 1963 destroyed septal walls of the tubificid at the site of infection (Calentine 1967). In the same study, Calentine found that much of the body wall musculature of L. hoffmeisteri was damaged or destroyed by developing larvae of M. hunteri. Calentine (1963) noted compression of the tubificid intestinal tract as a normal occurrence in L. hoffmeisteri harboring A. iowensis; complete obstruction was observed in one specimen. Additionally, the bodies of parasitized annelids were often distended and weakened at the site of infection.

Coelomic phagocytes found in certain oligochaetes may also play a role in host defenses to caryophyllidean infections (Calentine et al. 1970). Tubificids such as B. sowerbyi, T. tubifex, I. templetoni and L. hoffmeisteri have phagocytic cells, normally lining the septa and body

wall; these attack developing procercoids of a number of caryophyllideans. Very young procercoids are often completely surrounded by such cells. Certain caryophyllidean species seemed to be killed by these phagocytes; some were merely retarded in growth rate and others soon became free of host cells and developed into procercoids within normal time limits.

Size of the annelid appears to affect the caryophyllidean-oligochaete relationship. Because of their small size, naidid oligochaetes are usually not suitable hosts for a number of caryophyllideans (Calentine et al. 1970). Procercoid growth often causes rupture of the naidid body wall and subsequent death of the host. For example, Stylaria and Nais infected with G. confusa invariably perished before completion of procercoid development (Calentine et al. 1970). Calentine (1967) noted that rate of development of certain procercoids was retarded in smaller oligochaete species in contrast to larger species.

Another factor influencing the parasite pathology in the annelid is procercoid size (Calentine 1967). The smaller the procercoid, the less likely it will be to damage the host. Calentine found that the comparatively small larvae of H. nodulosa appeared to cause little damage to L. hoffmeisteri. In fact, some infected tubificids became sexually mature, a condition rarely observed in annelids

infected with larvae of other caryophyllidean species examined.

A final factor influencing oligochaete pathology, and yet closely related to procercoid size, is the intensity of procercoid infection. Calentine (1965) recovered as many as 55 Biacetabulum macrocephalum McCrae, 1962 procercoids from L. hoffmeisteri ten days following exposure to eggs, but by 101 days post-exposure the most heavily infected oligochaete harbored 11 parasites. Likewise, Calentine (1963) found as many as 18 A. iowensis in a single tubificid, 30 days post-infection. Examination of infected oligochaetes at later dates revealed substantial reductions in infection levels.

Pathology in Aulodrilus pigueti

As previously stated, A. pigueti appears to be the only suitable intermediate host for K. iowensis in the Little Sioux River. Since A. pigueti is a relatively small (2-28 mm in length) tubificid when compared with L. hoffmeisteri (20-35 mm in length), it would appear advantageous for completion of procercoid development if the infective stage of K. iowensis were relatively small (less than 1.2 mm in length). Nonetheless, the small procercoid of K. iowensis does some damage to A. pigueti. Septal walls of the

oligochaete are destroyed in the region of procercoid development. Because of mechanical pressure exerted by the growing procercoid, the septum bulges away from the larva and eventually ruptures. The region of the oligochaete (segments VIII through XIII) harboring infective procercoids is often distinctly swollen and the annelid body wall becomes more transparent than in corresponding areas of uninfected A. pigueti. Increase in girth of the oligochaete does not appear to be the result of hyperplastic growth, but rather results from distention of the existing body wall.

Examination of sectioned material reveals the body wall to be considerably thinner in infected oligochaetes than it is in uninfected hosts. Much of the body wall musculature is damaged and destroyed in the infected region. No compression of the intestinal tract or other effects of the oligochaete gut are evident. Examination of sectioned material of a chronological series of procercoid developmental stages did not reveal any phagocyte involvement. At no time during development of K. iowensis were any host cells found associated with the procercoid. Since the asexually reproducing A. pigueti normally produces only incipient reproductive structures, the possibility of sterilization by K. iowensis is moot.

Death of the tubificid was noted during experimental

studies. As many as nine procercoids were found within a single oligochaete, 30 days post-infection. However, after 60 days, tubificids harboring more than four larvae were not found. It was noted that the body wall of individual A. pigueti containing larger procercoids often was highly distended. During routine handling, these oligochaetes often ruptured in the region of distension, thereby liberating the procercoids. Liberated procercoids were initially very active, but usually became swollen and moribund and died within hours. Ruptured oligochaetes often died within hours, but several lived, surprisingly, for several weeks.

Fish Pathology Elicited by Caryophyllideans

In Europe and Asia, a number of caryophyllideans have long been recognized as producing disease symptoms in their fish hosts (Bauer 1959). In farmed carp, reports of diseases attributed to infection with K. sinensis (khawiasis) and C. fimbriceps (caryophyllosis) are widespread, especially in the Russian literature (Bauer et al. 1969). Outbreaks of khawiasis occur usually in May through June in carp ponds around Moscow. Two-year-old fish die only if infected by "some tens of parasites," whereas mortality of three-year-old carp results from infection with "about 45 large

parasites" (Bauer et al. 1969).

The clinical pathology of K. sinensis in carp, as summarized by Bauer et al. (1969), is reviewed below. Heavy infection with K. sinensis results in a marked swelling of the abdomen. The intestinal tract is also distended, the gut wall being so thin that the parasites are visible from its exterior surface. Perforation of the gut and intestinal obstruction may occur in particularly heavy infections. Infected fish become sluggish and emaciated; they swim close to the surface and cease to feed. Hemoglobin content of the blood is often significantly reduced. Heavy infections often prove fatal.

Macroscopic examination reveals that K. sinensis attached to the gut mucosa cause mechanical lesions and inflammation. If infection levels are moderate, there is "desquamative catarrhal enteritis, which later develops into catarrhal hemorrhagic enteritis." The inflamed mucosa is covered with exudate and becomes hyperemic and hemorrhagic. Occasionally, small ulcers with edematous, reddened margins develop.

Examination of sectioned material reveals edema and infiltration of blood cells. The mucosal epithelium is often degenerative and necrotic. The mucosa is eroded and the submucosa exposed. Connective tissue proliferation

occurs at the point of scolex attachment. The muscularis may also be affected. Blood vessel dilation and extravasation are common at the point of attachment. Inflammation is at first local but becomes diffuse in chronic infections. It is also maintained that K. sinensis secretes toxic substances that are absorbed by fish and which may cause degenerative changes of "some parenchymatous organs."

Caryophyllosis involves a clinical picture similar to that of khawiasis. Mortality is rare but fish lose weight and growth is retarded. One- and two-year-old carp are mainly affected. Bauer et al. cited a specific example of caryophyllosis in carp fry which initiated early benthic feeding because of the absence of zooplankton in the pond.

Banhawy et al. (1975) observed the effect on the ileum, liver and pancreas of the African silurid Synodontis schall (Bloch-Schneider) when infected with Wenyonia virilis Woodland, 1923. Examination of tissues of heavily infected fish revealed considerable degeneration of the mucosa and submucosa and concurrent disturbance of the muscularis and serosa. The submucosa became hemorrhagic in the region of attachment and mechanical pressure of the parasite body reduced the height of adjacent villi. Degenerative changes noted in the liver and pancreas were attributed to toxins produced by the parasite. Sircar and Sinha (1974) found that in Clarias batrachus (L.) infected with Lytocestus

indicus (Moghe, 1925), hemoglobin content of blood was reduced. There was also a high degree of eosinophilia, in addition to anisocytosis, macrocytosis and poikilocytosis of red blood cells. These features were thought to be suggestive of macrocytic pernicious anemia seen in mammals infected with certain cestodes.

Pathological effects of caryophyllideans of North American fish have been little examined because of the lack of economic importance of affected cyprinids and catostomids. Mackiewicz et al. (1972) studied at the light microscopy level the relationship of pathology to scolex morphology of 15 North American caryophyllideans. They determined that caryophyllideans with specialized scoleces having loculi, bothria or acetabula generally elicit little or no pathology; on the other hand, those with a terminal introvert, weakly developed loculi or lacking specialized structures usually elicit pronounced host-reactions in the form of nodules or shallow ulcers. It was found that species with similar types of scoleces attach in similar ways. An apparently mucoid interface layer is often present between host and parasite of some species.

Hayunga (1979) examined the structure of the host-parasite attachment interface of three caryophyllideans of white suckers. As with the results of Mackiewicz et al.

(1972), the caryophyllidean with the unspecialized scolex (H. nodulosa) elicited the most pronounced response, whereas the two species with well-developed loculi and acetabula produced minimal damage. Suckers infected with H. nodulosa showed disruption of the mucosa and chronic inflammation of the submucosa characterized by infiltration of lymphocytes and extensive hyperplasia. Examination of the host-parasite interface using electron microscopy revealed the existence in all three caryophyllideans of an amorphous layer of electron lucent material (termed the "eosinophilic matrix") which appeared to be adhesive; this matrix was found to be secreted by certain scolex glands.

Pathology in Cyprinus carpio

Over 500 carp infected with K. iowensis were examined using a dissecting microscope; few pathologic conditions were revealed. Even in fish infected with several hundred cestodes, no significant, discernible reaction was observed. Occasionally, the intestine of immature carp harboring gravid cestodes was slightly distended and large worms within the gut lumen could be seen outside the unopened gut. However, instances of intestinal distention, more pronounced than those observed in parasitized fish, were seen in uninfected young carp that had ingested large amounts of food material. Intestinal blockage of K. iowensis was

not observed, but in heavy infections of small fish, normal absorption and passage of gut contents may be hindered. Regardless of infection intensity or size of fish, hemorrhage and inflammation were never grossly observed.

The anterior lumen of the carp gut, instead of possessing intestinal villi, is lined by a series of low intestinal folds which anastomose and form a reticulated pattern when viewed from above. The normal mode of scolex attachment for K. iowensis involves placement of individual scolex frills into the depressions formed by adjacent intestinal folds. Great variation in the shape of scolex is readily seen if the intestinal tract is first fixed and the cestodes later removed. Careful removal of scoleces of living worms reveals only mechanical displacement of mucosal folds. Gross effects of such displacement disappear within minutes of removal of the parasite.

Examination of sectioned material reveals that K. iowensis does, in fact, attach between mucosal folds with some mechanical displacement and compression of mucosal epithelium (Fig. 6). In both light and heavy infections, no evidence of inflammation or ulceration was noted. There is some additional compression of the mucosal epithelium where the parasite strobila contacts the gut wall. In such cases, the normally obvious brush border of the mucosal epithelium is obscured; however, no apparent differences in the

appearance or number of epithelial goblet cells exist between infected and uninfected regions of the gut.

The contrast in pathogenicity between K. sinensis and K. iowensis is dramatic. It is possible that carp in nature do not acquire sufficiently heavy worm burdens of K. iowensis to elicit severe pathological reactions. Alternatively, wild young-of-the-year carp may acquire heavy cestode infections and are killed; such carp would not be represented in collections of this study. However, the relatively heavy infections seen in carp in the present study and the lack of injury noted, leads one to conclude that K. iowensis is of little epizootic significance in wild carp of Iowa. It is interesting that the literature contains no reference to the pathogenicity of K. japonensis.

SUMMARY AND CONCLUSIONS

1. The life cycle of Khawia iowensis has been examined experimentally and in naturally infected hosts collected in Iowa during 1977 through 1981. Although representatives of seven species of cyprinids and of eight species of catostomid fish were examined, K. iowensis was limited to carp (Cyprinus carpio). Eight hundred seventy three carp from five rivers and from four lakes in Iowa were examined; these cestodes were present in all but the Mississippi River where only one carp was examined. Khawia iowensis occurs coast to coast in North America and is probably found in Iowa wherever carp and suitable tubificid intermediate hosts occur.

2. Eggs shed by cestodes from carp are unembryonated and require about 17 days in water for oncospheres to develop. Oncospheres may remain viable for more than 100 days in water. Further development does not occur until embryonated eggs are ingested by a suitable tubificid host.

3. Even after examination of many thousands of oligochaetes, natural infections of larval K. iowensis were not found in the Little Sioux River or Lower Gar Lake. However, large suprapopulations exist in carp of both habitats. This indicates that the infection prevalence of oligochaetes with K. iowensis procercoids is extremely low.

4. Aulodrilus pigueti, Limnodrilus hoffmeisteri,

Ilyodrilus templetoni, Branchiura sowerbyi, Dero digitata and Stylaria lacustris were exposed to embryonated eggs of K. iowensis; infections occurred only in A. pigueti. During feeding experiments, 27% of A. pigueti acquired infections.

5. Hatching of the oncosphere and its penetration of the oligochaete's intestinal wall were not observed, but embryos were seen within the body cavity less than three hours after exposure to viable eggs. Evidence suggests that penetration occurs between segments XX and XXXII. Procercoids then migrate anteriad within the coelom, where development normally continues in segments VIII through XIII.

6. A cerocomere, bearing six embryonic hooks, is first evident at about day 40; the typically frilled scolex of the adult is evident by day 60. Procercoids do not become progenetic.

7. Experimental feedings of infected oligochaetes to carp did not give conclusive evidence concerning the manner by which fish acquire K. iowensis infections. However, there appears to be little doubt that carp become infected by ingesting infected tubificids. The failure of experimental feedings of cestodes to carp may reflect the use of non-infective procercoids in such experiments.

8. Carp infections of K. iowensis demonstrate a distinct periodicity. During August, prevalence may be as

high as 87%; as many as 800 worms may occur in a single host. During winter and early spring, incidence and mean intensity are at their lowest levels. No gravid cestodes were recovered during winter collections. This seasonal distribution appears closely correlated to seasonal variations in feeding activities of carp and concurrent natural senescence of worms during the later part of summer.

9. Carp infections of Capillaria sp. and Pomphorhynchus bulbocolli lack seasonal periodicity. Immature and gravid worms of both species were recovered from carp intestinal tracts during all seasons.

10. Site selection within the carp intestinal tract was noted for K. iowensis, Atractolytocestus huronensis, P. bulbocolli and Capillaria sp. Khawia inhabited the anterior half of the carp gut, A. huronensis the anterior quarter, P. bulbocolli the third quarter and Capillaria, in lightly infected hosts, the fourth quarter of the tract.

11. Cestodes may either be attached to the gut mucosa of carp or may occur free within the intestinal contents. Evidence suggests that unattached Khawia represent moribund or damaged worms that are being expelled from the host. The more posteriad site selection of gravid Khawia provides some evidence for an ontogenetic shift in attachment points.

12. The correlation coefficients calculated

between prevalence and/or mean intensity of K. iowensis with carp length were both positive for the Little Sioux River. Except for the relative scarcity of P. bulbocolli and Capillaria sp. from carp of less than ten cm in length, there is little correlation between worm burdens of the acanthocephalan or the nematode and fish length.

13. Male and female carp had similar prevalences and mean intensities of each helminth species.

14. Intestinal helminth populations of carp from lentic (Lower Gar Lake) and lotic (Little Sioux River) habitats were contrasted. Carp of the river had significantly higher worm burdens of K. iowensis. Scarcity of annelid hosts, annual migration of fish hosts, and possible differences in feeding activities probably led to the low infection levels of lake carp. Carp of the lake harbored more P. bulbocolli than did river carp. As contrasted to the lake, the river contained few amphipod intermediate hosts and few other suitable fish host species. The mean intensity of Capillaria sp. was significantly higher in Lower Gar Lake than in the Little Sioux River.

15. Members of the genus Khawia (K. iowensis, K. japonensis and K. sinensis) found in carp are compared and contrasted morphologically. Khawia sinensis is morphologically distinct from the other two species. However,

K. iowensis and K. japonensis possess few characteristics by which the two species can be separated. Until the status of L. udekemianus as an intermediate host of K. iowensis can be determined, it is suggested that K. iowensis and K. japonensis be retained as separate species.

16. The rather broad oligochaete host specificity of K. sinensis is in apparent contrast to the narrow specificity observed for K. iowensis and K. japonensis.

17. The relatively small procercoid of K. iowensis elicits destruction of the annelid host's septal walls and body musculature and causes distention of the outer body wall. Infected A. pigueti are easily ruptured in the region of procercoid development. No involvement of annelid phagocytes was observed during larval development. Compared with other caryophyllidean larvae, K. iowensis procercoids elicit little host injury.

18. Compared with the severe injuries caused by K. sinensis, adults of K. iowensis appear to be relatively benign in wild carp. The normal mode of scolex attachment involves placement of individual scolex fimbria into the depressions formed by adjacent intestinal folds of the carp gut. Only minor mechanical displacement and compression of mucosal epithelium was evident in heavily infected hosts. The normally obvious brush border of the mucosal epithelium

is obscured; however, no apparent differences in the appearance or number of epithelial goblet cells exist between infected and uninfected gut. In both light and heavy infections, no evidence of inflammation or ulceration was noted.

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PLATES

Abbreviations used in the illustrations to follow, are explained below:

C	Capillaria	oo	ootype
P	Pomphorhynchus	p	proboscis
a	anterior ovarian vitellaria	ph	pharynx
b	excretory bladder	pr	proboscis receptacle
c	cercomere	pv	postovarian vitellaria
ca	caudal papilla	r	retractor muscle
cb	copulatory bursa	s	spicule
cg	cement gland	sh	spicule sheath
cp	cirrus pouch	sp	spicule spine
e	esophagus	sr	seminal receptacle
eg	egg	st	stichocyte
eh	embryonic hooks	t	testis
ga	genital atrium	ta	testis anlagen
i	intestine	u	uterus
l	lemniscus	ua	uterine anlagen
ls	ligament sac	ub	uterine bell
n	neck	ug	uterine gland
nb	neck bulb	v	vagina
no	node	va	vitelline anlagen
nu	nucleus	vd	vas deferens
o	ovary	vu	vulva
ob	ovarian ball		

Plate I

Figures 1 through 3. *Khawia iowensis*, procercoids experimentally reared in *Aulodrilus pigueti* (Ages given in days, post-infection; scale as in Figure 3)

Figure 1. Procercoid at day 20

Figure 2. Procercoid at day 40

Figure 3. Procercoid at day 60

Figures 4 through 5. *Khawia iowensis*, immature worms recovered from naturally infected *Cyprinus carpio* (scale as in Figure 3)

Figure 4. Immature worm showing incipient testes, ovary and vitelline glands

Figure 5. Immature worm of minimal development

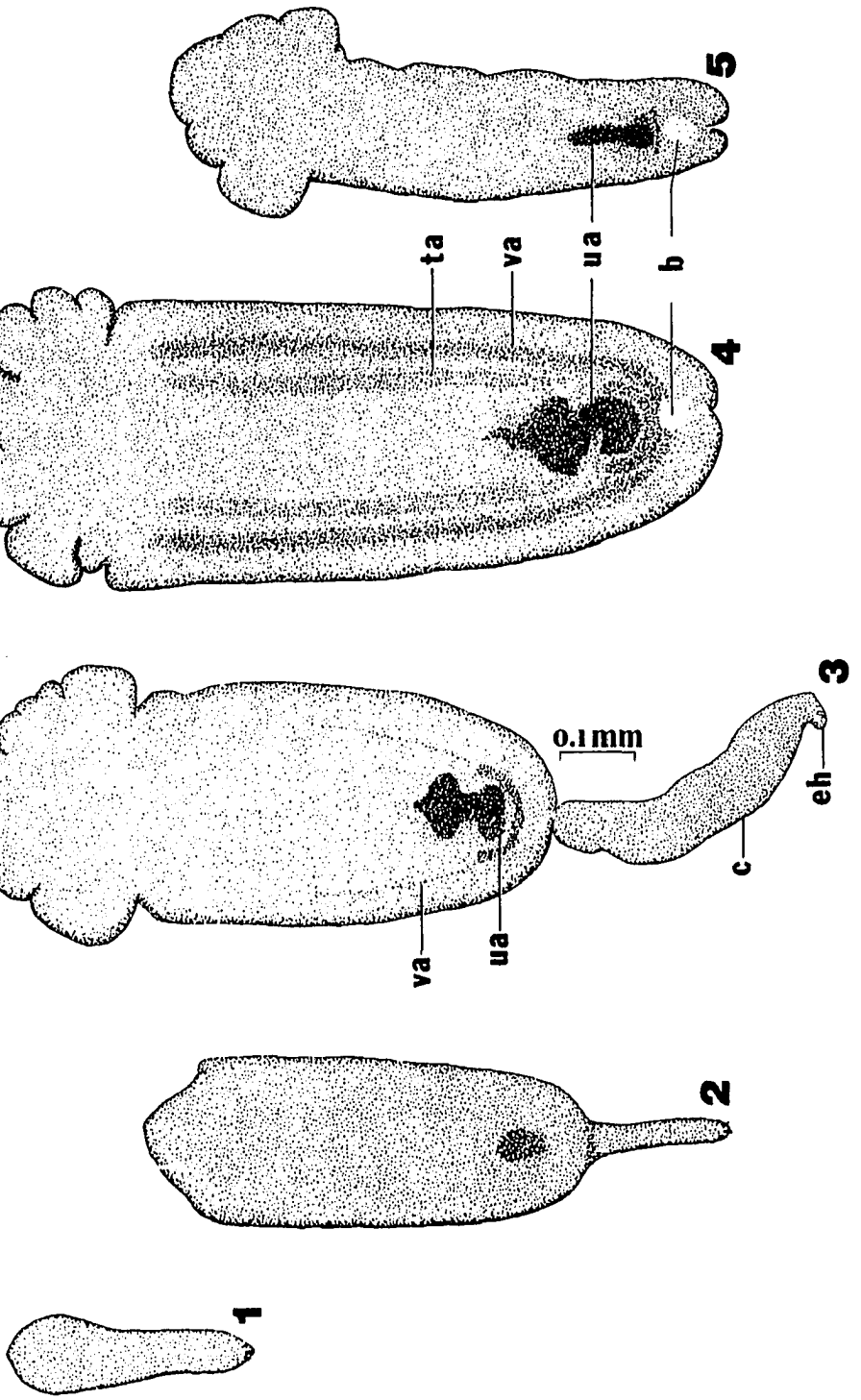
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Plate II

Figures 6 through 7. *Khawia iowensis*, adults attached to intestinal mucosa of *Cyprinus carpio*

Figure 6. Scolex attachment

Figure 7. Strobila of a single worm folded back upon itself two or three times

Figure 8. Orientation of *Pomphorhynchus bulbocolli* and *Capillaria* sp. within the intestine of *Cyprinus carpio*

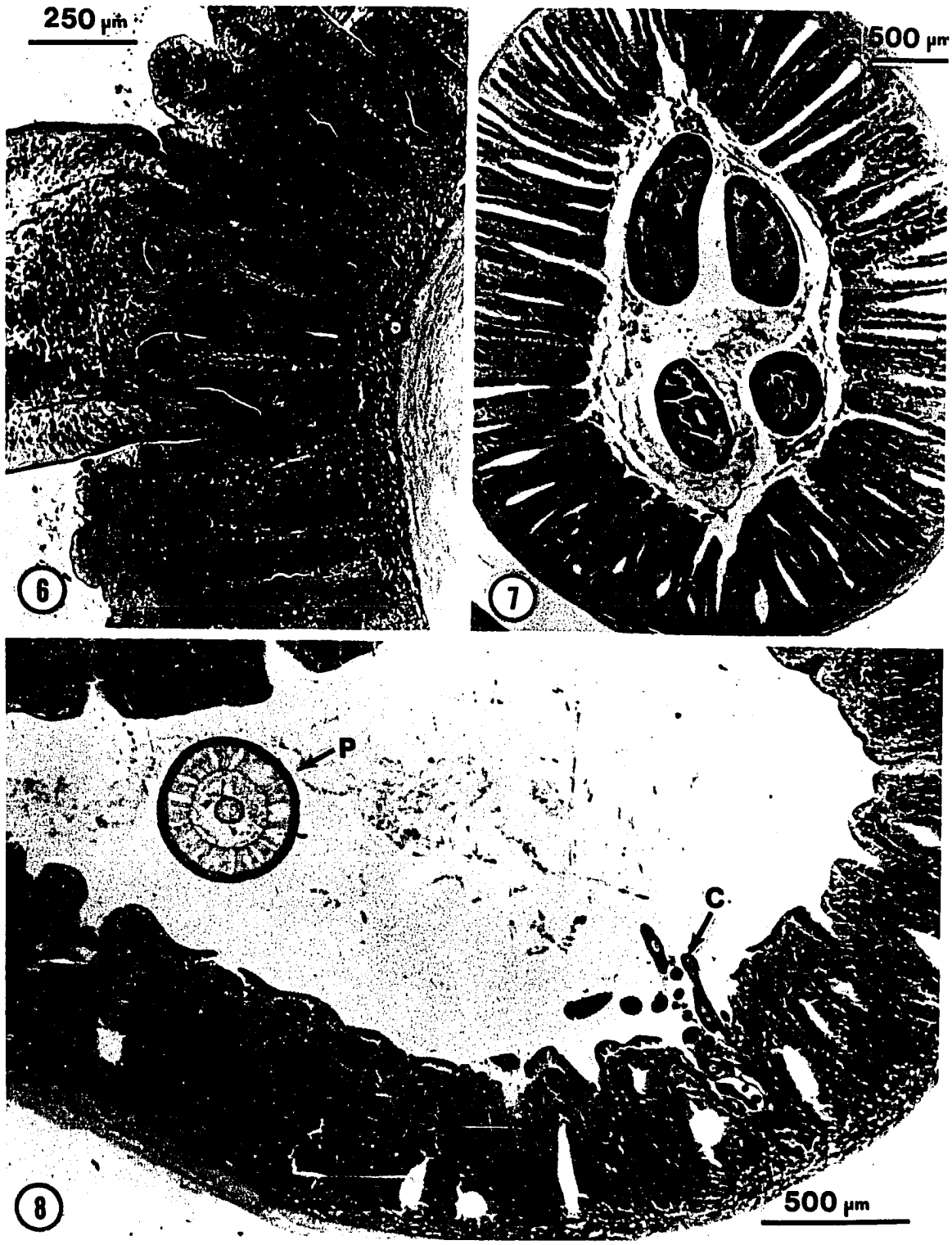
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plate III

Figures 9 through 12. *Khawia iowensis*, morphology of gravid adults from intestine of *Cyprinus carpio* (natural infections)

Figure 9. Gravid worm with typical scolex of cold formalin fixed material

Figure 10. Scoleces of cold formalin fixed (a) and hot formalin fixed (b through e) material

Figure 11. Excretory tubule network in testicular region of worm

Figure 12. Excretory bladder and excretory tubules in post-ovarian vitellaria region of worm

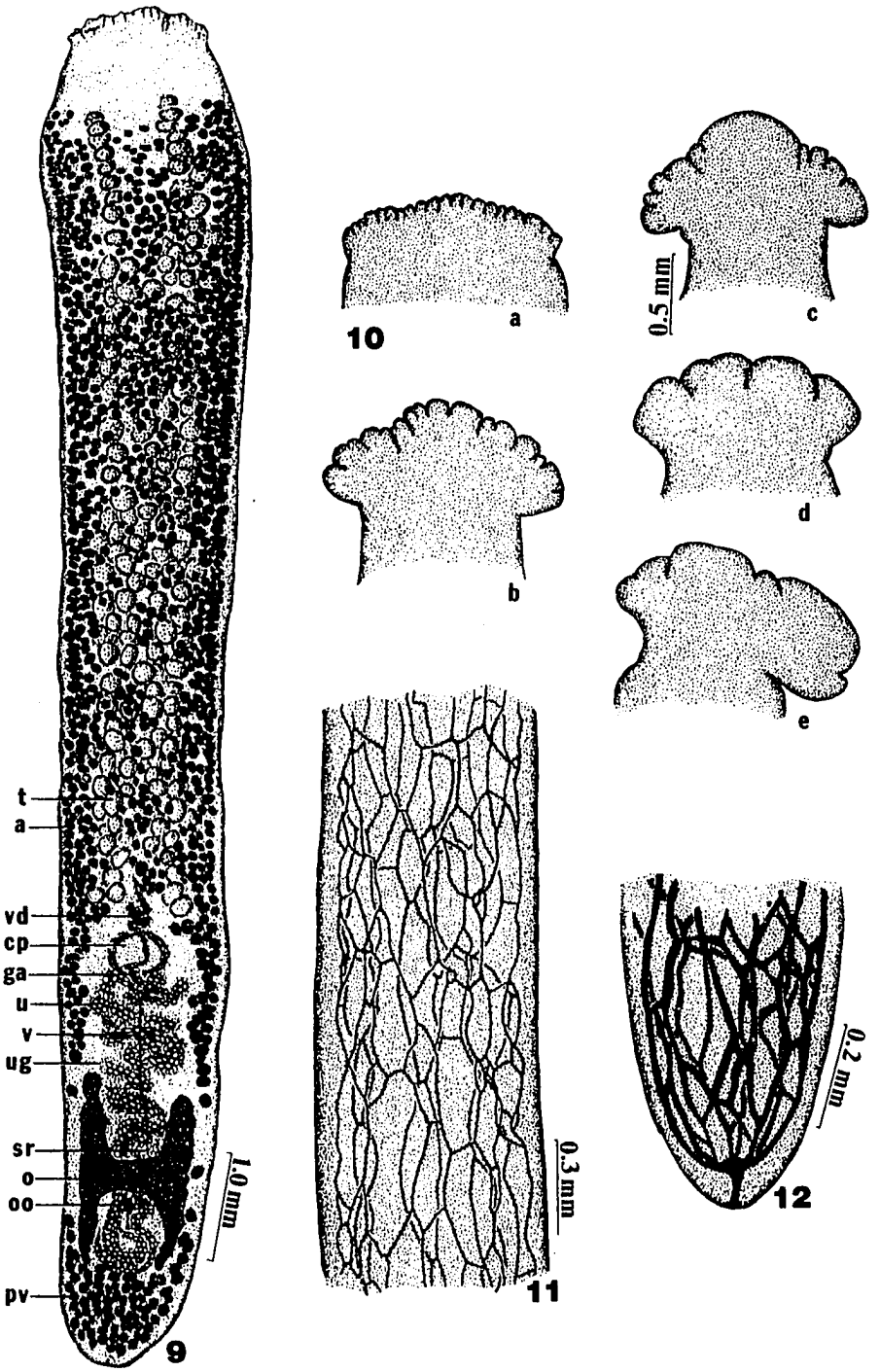
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Plate IV

Figures 13 through 19. *Capillaria* sp. from the intestine of *Cyprinus carpio*

Figure 13. Male (scale as in Figure 14)

Figure 14. Female

Figure 15. Pharynx, esophagus and stichosome of adult. Notice reduction in diameter of esophagus in the region of stichocytes

Figure 16. Stichosome and esophagus (scale as in Figure 15)

Figure 17. Vulvular region of female (scale as in Figure 15)

Figure 18. Posterior region of female (scale as in Figure 15)

Figure 19. Posterior region of male (scale as in Figure 15)

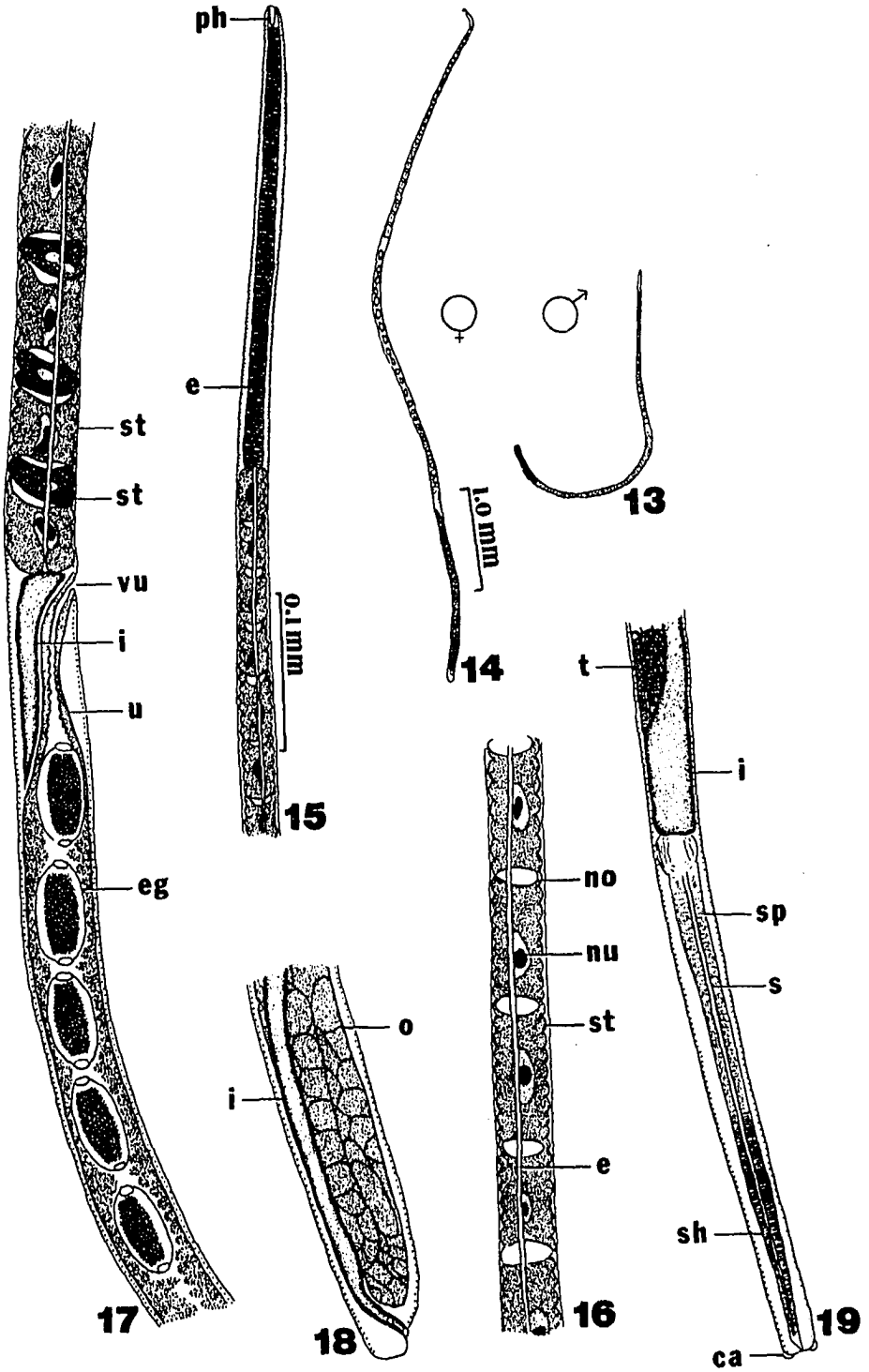
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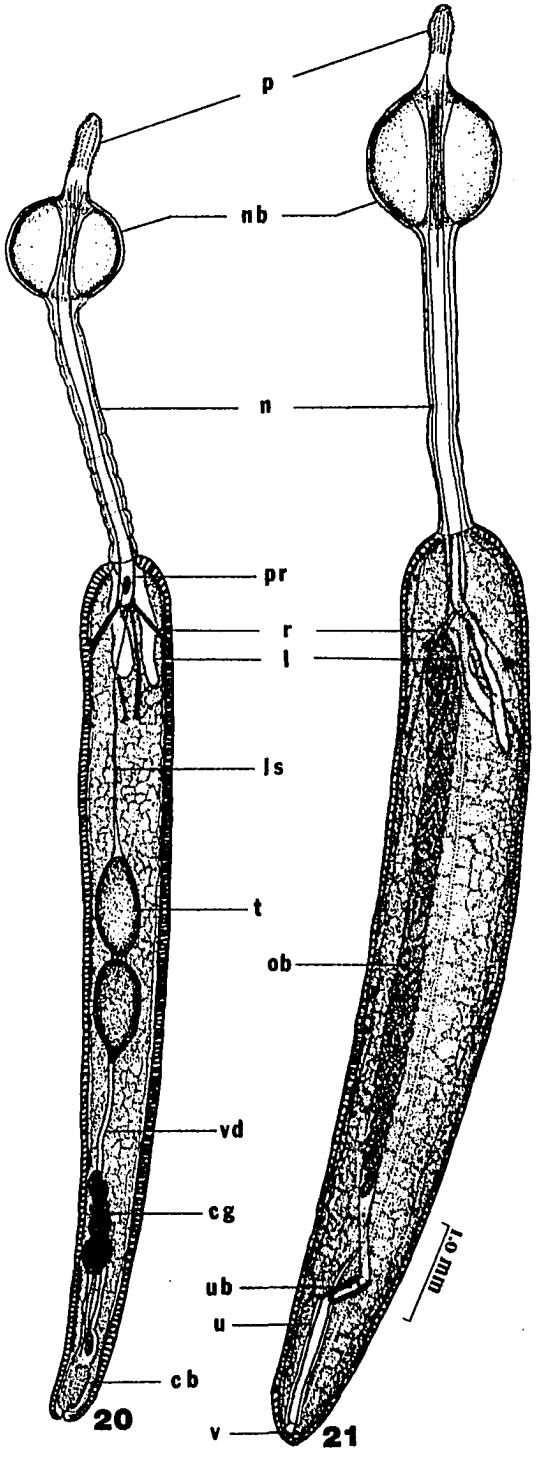
Plate V

Figures 20 through 21. *Pomphorhynchus bulbocolli* from intestine of *Cyprinus carpio* (natural infections)

Figure 20. Mature male (scale as in Figure 21)

Figure 21. Female at ovarian ball stage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